



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> NOVEL PEPTIDES FOR PREVENTION AND TREATMENT OF INFECTION  <b>(57) Abstract</b> <p>Peptides of 8 to 15 amino acids in length are described possessing neutrophil and/or monocyte/macrophage stimulatory activity. The peptides may be used in methods of treatment of various diseases and conditions in which enhancement of neutrophil and/or monocyte/macrophage function is desirable. The peptide is of the general formula: X<sub>1</sub>-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-Ser-Thr-X<sub>5</sub>-Val-X<sub>6</sub>-Ile-Thr-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub> in which, X<sub>1</sub> is absent, Cys or R<sub>1</sub>; X<sub>2</sub> is absent, Ala, Arg, Glu or Gly; X<sub>3</sub> is absent, Ala, Arg, Asn, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Trp, <math>\gamma</math>-Abu, <math>\beta</math>Ala, Dbu, Sar, Suc or N-Me-Ala; X<sub>4</sub> is absent, Ala, Arg, Asn, Glu, His, Leu, Lys, Met, Pro, Ser, Trp, <math>\beta</math>Ala or Nip; X<sub>5</sub> is Ala or His; X<sub>6</sub> is Ala, Gly, Ile, Leu, Phe, Pro, Ser, Thr, Trp, Val, D-Ala, D-Ile, D-Pro, D-Ser, D-Thr, D-Val or <math>\beta</math>Ala; X<sub>7</sub> is His or Ala; X<sub>8</sub> is absent, Ile, Leu, Thr or D-Ile; X<sub>9</sub> is absent, Ile, D-Ile or Aib; and X<sub>10</sub> is absent, Cys or R<sub>2</sub>.</p>		

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## NOVEL PEPTIDES FOR PREVENTION AND TREATMENT OF INFECTION

### Field of the Invention:

5 The present invention relates to peptides having neutrophil and/or monocyte/macrophage stimulating activity, and to the use of these peptides as therapeutic agents.

### Background to the Invention:

10 The phagocytic leukocytes include three types of granulocytes; neutrophils, eosinophils and basophils; and two types of mononuclear phagocytes; monocytes and macrophages. Neutrophils predominate in the response to acute bacterial and fungal infections; monocytes and macrophages respond to chronic infections, especially those caused by intracellular pathogens; eosinophils are associated with allergic reactions and  
15 invasion by metazoan parasites and basophils are associated primarily with immunologic disorders. The responses of phagocytic cells to invasion by microorganisms are mediated by humoral agents derived from both microbial (e.g. endotoxin, chemotactic factors) and host (e.g. antibodies, the complement system, cytokines) sources. These molecules bind to receptors  
20 on the phagocyte plasma membrane and thus trigger signal transduction elements that lead to functional cellular responses. Initial contact with certain mediators, e.g. tumour necrosis factor (TNF), results in priming of the neutrophil, (for example, through up regulation of receptors), so that subsequent challenge elicits an enhanced response.

25 Phagocyte defects are either acquired or inherited disorders and are associated with either inadequate numbers of circulating cells (neutropenia, granulocytopenia) or impaired function (such as in chronic granulomatous disease (CGD), myeloperoxidase deficiency, adherence glycoprotein deficiency, Chediak-Higashi syndrome, specific granule deficiency and Job's  
30 syndrome). Secondary failure of phagocyte responses occurs in disorders of humoral mediator systems, such as agammaglobulinemia or complement deficiency syndromes. The major clinical manifestation of phagocyte disorders is recurrent infections, most often involving the skin, lymph nodes, and respiratory tract. Frequently encountered pathogens include *S. aureus*,

*Streptococci*, gram negative bacilli, *Candida*, *Aspergillus* and *Nocardia*. Periodontal disease also has a high prevalence and poor wound healing is characteristic.

Various defects in neutrophil function have been described as  
5 acquired disorders in some patients with thermal injury, bacterial or viral  
infections (see below), rheumatoid arthritis, systemic lupus erythematosus,  
diabetes mellitus, malnutrition, disseminated malignancy, bone marrow  
transplantation, and a number of other problems including age (i.e. the  
10 elderly and neonates) and alcoholism. In particular, depressed neutrophil  
function is known to occur in a number of viral, fungal, bacterial and  
parasitic infections (Abramson and Mills, 1988), *Rev. Infect. Dis.* **10**; 326-341;  
Ferrante *et al.* 1989. *Immunol. Letts.* **22**; 301-6). In addition, depressed  
neutrophil function has been described in Acquired Immune Deficiency  
15 syndrome (Thorsen *et al.*, 1984. *AIDS.* **3**; 651-653; Ellis *et al.* 1988, *J. Infect.*  
*Dis.* **158**; 1268-1276; Murphy *et al.*, 1988. *J. Infect. Dis.* **158**; 627-630), cancer  
patients, (who may also suffer neutropaenia as a result of radio and  
chemotherapy, consequently leaving them more susceptible to viral, bacterial  
and fungal infections: Bodey *et al.*, 1966. *Ann. Intern Med.* **64**; 328), and  
20 patients with acute leukemia undergoing bone marrow transplant (especially  
those with depleted T cell subsets, who are particularly at risk of fungal  
infection: Pirsch and Maki, 1986. *Ann. Intern. Med.* **104**; 619-631). Also, it is  
known that improved phagocytic cell function may protect against infection  
in graft vs host disease and in patients treated with cyclosporin A (Kennedy  
*et al.* 1994. *J. Clin. Oncol.* **12**; 249-57).

25 In patients with phagocyte dysfunction syndromes and conditions,  
early recognition and aggressive treatment of infections may be crucial.  
Unfortunately, microbial resistance to antibiotics is an increasing world-wide  
problem, thus new approaches to therapy are required. Immunotherapy has  
the advantage that the body's own defenses are upregulated, potentially  
30 leading to a reduction in antibiotic use. For example, interferon- $\gamma$ , a  
macrophage stimulating agent, decreases the frequency of serious infections  
in patients with CGD.

CONDITIONS AND DISEASES CAUSED BY BACTERIA:

Agents which upregulate the ability of neutrophils and/or monocytes/macrophages to treat bacterial infection would be desirable.

5       Tuberculosis presents a major health problem throughout the world  
with a recent resurgence of tuberculosis in industrialised societies (Snide *et al* IN: Bloom B R, ed. Tuberculosis Washington; ASM Press, 1994: 3-12; Friedman *et al* 1996. Engl. J. Med. 334; 828-833; Frieden *et al*, 1993. N. Engl. J. Med. 328; 521-526). The greatest risk factor for tuberculosis is coincident  
10       infection with Human Immunodeficiency virus (HIV) which destroys CD4  
positive T cells which act to control infection. Immunosuppression by either  
drug therapy or HIV co-infection also allows recrudescence of infection in a  
person who had previously controlled the infection. Advanced HIV infection  
is also complicated by infection with environmental mycobacteria of the  
15       *Mycobacterium avium* complex (MAC). Infection with MAC is difficult to  
treat because of its resistance to multiple antibiotics. Agents which  
stimulate the host response to mycobacteria would be advantageous as they  
may shorten the period of chemotherapy necessary to eradicate the organism.  
They may also permit new approaches to the treatment of infection with drug  
resistant *M. tuberculosis* and *M. avium*.

20       In most patients with recurrent infections however, no white blood  
cell defect, complement or immunoglobulin abnormalities can be identified.  
Frequently, breaks in the skin from burns, scratches, bites and surgical  
wounds allow bacteria to bypass the normal defense of intact skin. Many  
pathogenic bacteria are carried by normal individuals, e.g. up to 30% of  
25       healthy people carry *S. pneumoniae* and up to 40% of healthy people carry *S.*  
*aureus*. Pneumonia caused by these organisms may arise during viral  
respiratory infection. Similarly, in chronic obstructive pulmonary disease  
(COPD), such as chronic bronchitis, the initial insult, resulting in increased  
cough and expectoration, is probably viral. While no infective organism is  
30       found in all patients, *Haemophilus influenzae* and *Streptococcus pneumoniae*  
are the most commonly cultured organism. In addition, *Mycoplasma* is  
occasionally present. A growing body of evidence relates recurrent  
childhood respiratory infections to COPD in later life (Higgins 1984, Chest  
85(Suppl.):35).

35       Most community acquired pneumonia's are due to *S. pneumoniae*.  
However, *Haemophilus influenzae*, once considered a relatively infrequent

cause of adult pneumonia, is now estimated to cause 2% to 20% of acute community acquired pneumonia. Furthermore, up to 15% of cases of community acquired pneumonia in the United Kingdom is caused by *Legionella pneumophila*. Marginally compromised hosts such as nursing  
5 home patients, elderly alcoholic patients, and the chronically disabled at any age, represent an important subset of patients in whom the cause of community acquired bacterial pneumonia differs from that seen in younger, normal populations. *S. aureus* and gram negative bacilli including *H. influenzae*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* are more  
10 common causes of pneumonia in this group.

Group B Streptococci is a common cause of severe neonatal infections, including pneumonia and meningitis. Host defense, which is particularly lacking in neonates, against Group B Streptococci depends entirely on the phagocytic and oxygen-dependent killing of the organisms by  
15 neutrophils.

*Legionella pneumophila* is a facultative gram-negative bacterium that has been identified as the etiologic agent of Legionnaires disease. *Legionella pneumonia* is believed to follow the inhalation of contaminated water aerosols. The bacteria are ingested by the resident alveolar macrophages  
20 within which Legionellae multiply. The multiplying bacteria eventually disrupt the alveolar macrophages and the released bacteria are in turn phagocytosed by mononuclear phagocytes and neutrophils recruited from the circulation. As mentioned above, *Legionella* may cause up to 15% of community acquired pneumonia as well as 1-40% of nosocomial  
25 pneumonias. TNF $\alpha$  has been shown to protect mice against lethal *Legionella pneumophila* infection via activation of neutrophil function (Blanchard *et al.*, 1988 J Leukocyte Biol. 43:429) while interferon- $\gamma$  treatment of human monocytes inhibits its growth *in vitro*.

Patients with Cystic Fibrosis (CF) are particularly vulnerable to  
30 bacterial colonization of the lung despite apparently normal phagocytic cell function and cell-mediated immunity. Over 95% of the deaths of CF patients are caused by lung diseases. At birth, before infection, the lungs are histologically normal. Usually early in life, lung infection occurs and obstruction of the small airways by mucus, inflammatory infiltrates, and  
35 mucosal oedema begins. Early in the course, bacterial infection becomes established and can not be eradicated despite prolonged and intensive

antibiotic therapy. *S. aureus* predominates early in the course but later *Pseudomonas aeruginosa* becomes predominate. *H. influenzae* and *Pseudomonos cepacia* are also common. Mycobacterial infection is rare. Specific immunotherapy as an adjunct to antibiotic therapy may be useful in  
5 reducing antibiotic use and hence prevent or delay the emergence of antibiotic resistant strains.

Most chronic bacterial skin infections occur in lower extremities and result from secondary infection of a break in the skin associated with a traumatic injury or underlying disease. The most common underlying  
10 diseases are diabetes mellitus, arterial insufficiency, venous stasis, vasculitis and haematologic problems such as sickle cell anaemia, leukemia and dysproteinemias. Most local skin infections are caused by *S. aureus*, *S. epidermidis*, and gram negative bacilli such as *P. aeruginosa* and *Aeromas hydrophila*.

15 Bacteremia occurs in both community and hospital acquired infections. Community-acquired infections commonly associated with blood stream invasion include pneumonia, pyelonephritis, meningitis and those associated with perforating injuries of abdominal and thoracic viscera and obstructing gastrointestinal or genitourinary tract lesions. Patients with soft  
20 tissue infections with diabetes, malnourished persons, and those receiving corticosteroid therapy or other forms of immunosuppression are also at risk. One of the most serious complications of bacteremia, especially in association with gram negative infections is circulatory collapse (septic shock).

25 Infections with *Listeria monocytogenes* cause a wide variety of distinct clinical syndromes. The most frequent are meningoencephalitis, primary bacteremia without meningitis and perinatal listeriosis. Endocarditis, isolated infection of the brain and other parenchymal organs, skin pustules and ocular infections are less frequent. The gastrointestinal  
30 tract is considered to be the portal of entry. Penetration of the intestinal villi, extension to the villous stroma, and ingestion and survival in non-immune macrophages provide access to the blood stream. Studies of listeriosis in murine models suggest that resistance occurs in two phases. Bacterial multiplication is limited by nonimmune macrophages and neutrophils in the  
35 first phase and by immune macrophages in the second. The microbiocidal activity of macrophages is associated with increased MHC following contact

with interferon- $\gamma$ . TNF, interleukin-1 (IL-1) and interleukin-2 (IL-2) also promote murine resistance. Those at risk of *Listeria* infection include patients immunosuppressed by lymphoreticular malignancies, immunosuppressive drugs (e.g. corticosteroids, cyclosporin A and estrogens) and pregnancy. Neonates and the elderly are also susceptible to *Listeria* infection.

#### FUNGAL AND PROTOZOAL DISEASES AND CONDITIONS:

Up-regulation of phagocytic function may be of assistance in the treatment of fungal and protozoal diseases and conditions.

The dimorphic fungus *H. capsulatum* is an intracellular parasite that infects the host by deposition of microconidia into the terminal bronchioles and alveoli of the lungs. Inhaled microconidia convert into yeasts that are phagocytosed by alveolar macrophages within which they multiply. Dividing yeasts destroy the alveolar macrophages and then are ingested by other resident alveolar macrophages or monocytes recruited to the loci of infection. Organisms may multiply intracellularly for 2 to 3 weeks before the development of cell mediated immunity (specifically armed macrophages), kill the fungus with the production of intense inflammation at the sites of infection. Like tuberculosis, necrosis occurs, and after several years the lesions may calcify. Where the development of cell-mediated immunity is delayed or fails to occur, progressive disseminated histoplasmosis occurs generally in individuals infected with HIV, taking glucocorticoids or other immunosuppressants or ongoing therapy for hematologic malignancies. However, apparently normal adults can also experience dissemination.

Cryptococcosis is an aerosol-borne mycosis. Of significance is the lack of any known host defect or pre-disposing condition in about half of all patients with cryptococcosis. Factors pre-disposing to cryptococcal infections include AIDS, lymphoma, corticosteroids, sarcoidosis, diabetes, cytotoxic drug therapy for cancer, and renal transplantation. Suppression of the neutrophil and macrophage activating cytokines interferon  $\gamma$  and TNF $\alpha$  increases susceptibility in a murine model of *Cryptococcus neoformans* infection reducing the ability of mice to survive central nervous system infection. (Aguirre *et al.*, 1995, *Infection and Immunity*, 63: 1725-1731). Further, it has been demonstrated that administration of recombinant human



TNF $\alpha$  protects mice from lethal *C. neoformans* lung infection (Kawakami *et al.*, 1996, Clin Exp Immunol 106: 468:474).

5 The causative agents of human norcardiosis are members of the Actinomycetaceae. *N. asteroides* is the most common species and is responsible for over 90% of pulmonary disease, disseminated disease or brain abscess. *N. brasiliensis* is responsible for 60% to 80% of primary skin infections. The basic tissue inflammatory response involves the neutrophil. Cell-mediated immunity and macrophage function are also considered to be important in the pathogenesis of the disease. Amongst other organisms  
10 responsible for skin infections are other fungi e.g. *Candida*, *Aspergillus*, and *Phycomycetes*. Superficial chronic fungal infection of the skin is a common problem but invasion from the skin rarely occurs. Fungi that produce nodular ulcerative skin lesions include blastomycosis, coccidioidmycosis and cryptococcosis.

15 The dygenetic protozoan *Trypanosoma cruzi* is the etiological disease of Chagas' Disease in man. The parasite infects a variety of host cell types including macrophages. Intracellular replication as amastigotes is followed by the release of trypomastigotes that can eventually reach the blood stream before infecting other host cells. Control of parasite load and host survival  
20 depend on effective T-cell mediated immunity via the cell-dependent protective antibody responses, macrophage activation for intracellular killing of the protozoan, and class I-dependent effector mechanisms. Cytokines synthesized in the course of the inflammatory and/or immune responses can regulate *in vitro* killing of the parasite by macrophages. The addition of  
25 TNF $\alpha$  or interferon  $\gamma$  to cultures of *T. cruzi* - infected macrophages results in more efficient killing of amastigotes by the phagocytes. Treatment of animals in a murine model of *T. cruzi* infection with either anti-interferon  $\gamma$  or anti-TNF $\alpha$  antibodies has been shown to increase levels of parasitemia (Abrahamson and Coffman, 1996, Exp Parasitol, 84: 231-244).

30 *Pneumocystis carinii* has been regarded as a protozoan parasite that is passed from human to human by the respiratory route, however, more recent evidence suggests that it is a fungus. *P. carinii* is usually not pathogenic until it has the opportunity to multiply and invade in a person who has a helper T cell defect.

VIRAL DISEASES AND CONDITIONS:

Agents which upregulate the ability of neutrophils and/or monocytes/macrophages to kill virus-infected cells may also be beneficial.

For example, in herpes simplex type I, following resolution of acute infection the immune response declines during the period of latency allowing the virus to be re-activated (Cantin *et al.*, 1995, J. Virology, 69: 4898-4905). Further the role of TNF $\alpha$ -mediated activation of macrophages in controlling acute herpes simplex virus infection has been established (Heise *et al.*, 1995, J. Virology, 69: 904-909).

Influenza A infects alveolar macrophages and infection leads to a comparatively slow development of a specific immune response, i.e. antibody production and generation of cytotoxic T lymphocytes. Therefore upregulation of macrophage function is particularly beneficial in the early phase of infection.

Neutrophils are the blood cells from which Cytomegalovirus (CMV) can usually be recovered in culture during viremia, although monocytes are also infected. CMV is a member of the herpesvirus family and infections with CMV are extremely common, occurring throughout life. Similar to other herpesviruses, after primary infection CMV remains in the host in a latent state. Infection of the immunocompetent host does not elicit severe clinical symptoms, however infection of the immunocompromised host can cause severe and even fatal disease, mainly CMV pneumonia. Morbidity and mortality associated with primary CMV infection or with reactivation from latency is common in immunosuppressed transplant recipients and in patients with immunodeficiency caused by HIV infection. Chemotherapy with the guanosine analog 9-(1,3-dihydroxy-2-propoxymethyl) guanine (ganciclovir) can limit viral spread but is associated with serious side effects principally neutropenia. In absence of an improvement of the immunological status, maintenance therapy with ganciclovir does not eliminate the virus and even progression of infection is possible. Combined prophylactic therapy with interferon- $\gamma$  and TNF $\alpha$  significantly reduces mortality in a murine model of CMV infection (Anderson *et al.*, 1993. Antiviral Research 21:343).

NOSOCOMIAL INFECTION:

In addition, the incidence and/or severity of nosocomial infections may be reduced by immuno therapy with peptides such as those described herein.

- 5           Hospital-acquired infections involve most commonly the urinary tract, biliary tract, pneumonia and wound infections.

*Candida* sp is emerging as a highly important nosocomial pathogen, the two most important pre-disposing factors are exposure to broad-spectrum antimicrobial agents and neutropenia. Neutropenia is not however a pre-  
10       requisite for disseminated candidiasis. About half of cases occur in patients with complicated post-operative courses unrelated to underlying neoplastic diseases.

*P. aeruginosa* accounts for approximately 10% of all hospital acquired infections. It is the second most common cause of nosocomial pneumonia,  
15       third leading cause of urinary tract infection and the fourth most common cause of surgical wound infections.

          Methicillin-resistant *S. aureus* is a common cause of nosocomial disease. *S. aureus* is the cause of hospital acquired pneumonia in approximately 10% of cases.

20       Wound infection follows urinary tract infection as the second most frequent type of nosocomial infection. In general, surgical wound infections that develop within 24 to 48 hours are due to group A  $\beta$  haemolytic Streptococci or *Clostridium perfringens*, whereas *S. aureus*, *S. epidermidis*, and gram negative bacilli wound infections occur 4 to 7 days after surgery.

25       Pressure scores (decubitus ulcers) are frequently nosocomial and are usually infected with two or more bacterial species.

          The present inventors have previously identified peptides derived from the primary amino acid sequence of human tumour necrosis factor  
30       (TNF) which stimulate neutrophil activity. These peptides which are described in Australian patent specification Nos. 74762/91 and 44664/93 (the disclosures of which are to be regarded as incorporated herein by reference), are of considerable clinical significance since treatment with such peptides is expected to enhance neutrophil and monocyte/macrophage activity, but  
35       would not be expected to cause the severe side effects associated with therapeutic use of the whole TNF molecule.

The present inventors have now identified further peptides exhibiting one or more improved properties over the abovementioned peptides. In particular, the present inventors have identified a "core" sequence within the previously described TNF-derived peptide 419 (PSTHVLITHTI), which  
 5 possesses neutrophil and monocyte/macrophage stimulatory activity. The core sequence (STXVXITX) is predicted to exhibit approximately 40% of the activity of peptide 419. Further, variation of this sequence has led to the identification of classes of peptides which have neutrophil stimulatory activity ("class 1"), equal neutrophil and monocyte/macrophage stimulatory  
 10 activity ("class 2"), or preferentially enhanced monocyte/macrophage stimulatory activity ("class 3"). Peptides in class 3 typically retain neutrophil stimulatory activity which may either be equal or greater than that displayed by peptides in class 1 or class 2 with exceptions as indicated below.

15 **Disclosure of the Invention:**

Thus, in a first aspect, the present invention provides a peptide with neutrophil and/or monocyte/macrophage stimulatory activity, wherein the peptide is of the general formula:-



20 in which,

$X_1$  is absent, Cys or  $R_1$ ,

$X_2$  is absent, Ala, Arg, Glu or Gly,

$X_3$  is absent, Ala, Arg, Asn, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Trp,  $\gamma$ -Abu,  $\beta$ Ala, Dbu, Sar, Suc or N-Me-Ala,

25  $X_4$  is absent, Ala, Arg, Asn, Glu, His, Leu, Lys, Met, Pro, Ser, Trp,  $\beta$ Ala or Nip,

$X_5$  is Ala or His,

$X_6$  is Ala, Gly, Ile, Leu, Phe, Pro, Ser, Thr, Trp, Val, D-Ala, D-Ile D-Pro, D-Ser, D-Thr, D-Val or  $\beta$ Ala,

30  $X_7$  is His or Ala,

$X_8$  is absent, Ile, Leu, Thr or D-Ile,

$X_9$  is absent, Ile, D-Ile or Aib, and

$X_{10}$  is absent, Cys or  $R_2$ ,

35  $R_1$  is H or R-CO, where R is H, straight, branched or cyclic alkyl up to C20, optionally containing double bonds and/or substituted with halogen, nitro, amino, hydroxy, sulfo, phospho or carboxyl groups which may be substituted

themselves or aralkyl or aryl optionally substituted as listed for the alkyl or R<sub>1</sub> is glycosyl, nucleosyl or lipoyl and R<sub>1</sub> is absent when the amino acid adjacent is an unsubstituted desamino-derivative; R<sub>2</sub> is -NR<sub>12</sub>R<sub>13</sub>, wherein R<sub>12</sub> and R<sub>13</sub> are independently H, straight, branched or cyclic alkyl, aralkyl or aryl optionally substituted as defined for R<sub>1</sub> or R<sub>2</sub> is N-glycosyl or N-lipoyl, or R<sub>2</sub> is -OR<sub>14</sub>, where R<sub>14</sub> is H straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for R<sub>1</sub> or R<sub>2</sub> is -O-glycosyl, or -O-lipoyl or R<sub>2</sub> is absent when the adjacent amino acid is a dicarboxy derivative of cysteine or a homologue thereof or the peptide is in a N-C cyclic form; with the proviso that the peptide is not Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile.

$\gamma$ Abu represents  $\gamma$ -aminobutyric acid, Dbu represents diaminobutyric acid, Aib represents amino isobutyric acid, Nip represents nipocotic acid,  $\beta$ Ala represents  $\beta$ -alanine, Sar represents sarcosine (alternatively known as N-methyl glycine), Suc represents succinic acid, N-Me-Ala represents N-methyl alanine.

The peptides according to the present invention may exhibit one or more improved properties over the peptides described in Australian patent specification Nos. 74762/91 and 44664/93. The improved property or properties may be increased potency, extended *in vivo* half life or, particularly, specificity of action.

In preferred embodiments of the invention, X<sub>1</sub> and X<sub>10</sub> are absent. X<sub>6</sub> is preferably Ile.

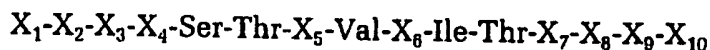
Particularly preferred peptides include:-

- (923) Ser-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,
- (925) Pro- $\beta$ Ala-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,
- (926)  $\beta$ Ala-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,
- (927) Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,
- (933) Suc-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,
- (934) Nip-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,
- (966)  $\beta$ Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,
- (967) Pro- $\beta$ Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,
- (968) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,
- (1059) Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,

- (1060) Lys-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1061) His-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1062) Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1063) Leu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
5 (1064) Trp-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1065) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1066) Asn-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1067) Glu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1068) Arg-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
10 (1069) Lys-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1070) His-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1071) Ala-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1072) Arg-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1073) Lys-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
15 (1074) His-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1075) Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1076) Leu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1077) Trp-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1078) Met-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
20 (1079) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1080) Asn-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1081) Glu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1083)  $\gamma$ Abu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1084) Dbu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
25 (1085) Sar-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1086) N-Me-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1087) Arg-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1088) Ala-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1089) Gly-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
30 (1090) Glu-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1097) Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1100) Pro-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
(1105) Pro-Ser-Thr-His-Val-Ile-Ile-Thr-Ala-Thr-Ile,  
(1108) Pro-Ser-Thr-His-Val-D-Ile-Ile-Thr-His-Thr-Ile,  
35 (1109) Pro-Ser-Thr-His-Val-Phe-Ile-Thr-His-Thr-Ile,  
(1161) Ser-Pro-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,

- (1162) Ser-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 (1163) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 (1164) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 (1165) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile,  
 5 (1166) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile,  
 (1168) Met-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1169) Met-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 (1170) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 (1171) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 10 (1172) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile,  
 (1173) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile,  
 (1175) Nip-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1176) Nip-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 (1177) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 15 (1178) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 (1179) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and  
 (1180) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

In a second aspect, the present invention provides a peptide with  
 20 neutrophil stimulatory activity (i.e. inactive or only negligibly active on  
 monocytes/macrophages), referred to herein as a class 1 peptide, wherein the  
 peptide is of the general formula:-



in which,

- 25  $X_1$  is absent, Cys or  $R_1$ ,  
 $X_2$  is absent,  
 $X_3$  is Ala, Lys or Ser,  
 $X_4$  is Pro,  
 $X_5$  is Ala or His,  
 30  $X_6$  is Ile or Leu,  
 $X_7$  is Ala or His,  
 $X_8$  is Ile, Thr or D-Ile,  
 $X_9$  is Ile or D-Ile,  
 $X_{10}$  is absent, Cys or  $R_2$ ,

35 wherein  $R_1$  and  $R_2$  are as defined above.

Particularly preferred class 1 peptides are:-

- (1073) Lys-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1075) Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1079) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 5 (1161) Ser-Pro-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1162) Ser-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 (1163) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 (1164) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 (1165) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and  
 10 (1166) Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

In a third aspect, the present invention provides a peptide with neutrophil and monocyte/macrophage stimulatory activity, referred to herein as a class 2 peptide, wherein the peptide is of the general formula:-

15  $X_1-X_2-X_3-X_4$ -Ser-Thr- $X_5$ -Val- $X_6$ -Ile-Thr- $X_7$ - $X_8$ - $X_9$ - $X_{10}$

in which,

- $X_1$  is absent, Cys or  $R_1$ ,  
 $X_2$  is absent,  
 $X_3$  is absent, Asn, Lys or  $\beta$ Ala  
 20  $X_4$  is Arg, His, Lys, Pro, Trp, Ala or Nip,  
 $X_5$  is Ala or His,  
 $X_6$  is Ile or Leu,  
 $X_7$  is Ala or His,  
 $X_8$  is Ile, Leu, Thr or D-Ile,  
 25  $X_9$  is Ile or D-Ile,  
 $X_{10}$  is absent, Cys or  $R_2$ ,

wherein  $R_1$  and  $R_2$  are as defined above; with the proviso that when  $X_3$  is Lys, then  $X_4$  is not Pro.

Particularly preferred class 2 peptides are:-

- 30 (966)  $\beta$ Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (968) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1060) Lys-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1061) His-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 35 (1062) Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1064) Trp-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,



(1069) Lys-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1080) Asn-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1175) Nip-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1176) Nip-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 5 (1177) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 (1178) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 (1179) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and  
 (1180) Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

10 Class 1 and 2 peptides may exert their stimulatory activity by, for example, eliciting superoxide production by neutrophils or by enhancing respiratory burst following treatment with N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP).

15 In a fourth aspect, the present invention provides a peptide with preferential monocyte/macrophage stimulatory activity (i.e. with enhanced monocyte/macrophage stimulatory activity as compared to class 1 and 2 peptides), referred to herein as a class 3 peptide, wherein the peptide is of the general formula:-

$X_1-X_2-X_3-X_4$ -Ser-Thr- $X_5$ -Val- $X_6$ -Ile-Thr- $X_7-X_8-X_9-X_{10}$

20 in which,

$X_1$  is absent, Cys or  $R_1$ ,

$X_2$  is absent, Ala, Arg, Glu or Gly,

$X_3$  is absent, Ala, Arg, Glu, His, Leu, Met, Trp,  $\gamma$ -Abu, Dbu, Sar or N-methyl Ala,

25  $X_4$  is Arg, Asn, Glu, Leu, Met or Pro,

$X_5$  is Ala or His,

$X_6$  is Ile or Leu,

$X_7$  is Ala or His,

$X_8$  is Ile, Leu, Thr or D-Ile,

30  $X_9$  is Ile or D-Ile,

$X_{10}$  is absent, Cys or  $R_2$ ,

wherein  $R_1$  and  $R_2$  are as defined above; with the proviso that when  $X_2$  is absent and  $X_3$  is Ala, then  $X_4$  is not Pro.

Particularly preferred class 3 peptides are:-

35

(1059) Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,

- (1063) Leu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1065) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1066) Asn-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1067) Glu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 5 (1068) Arg-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1070) His-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1072) Arg-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1074) His-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1076) Leu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 10 (1077) Trp-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1078) Met-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1081) Glu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1083)  $\gamma$ Abu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1084) Dbu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 15 (1085) Sar-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1086) N-methyl Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1087) Arg-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1088) \*Ala-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1089) Gly-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 20 (1090) \*Glu-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1168) Met-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 (1169) Met-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 (1170) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 (1171) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 25 (1172) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and  
 (1173) Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

Peptides in this class 3 generally display 70-130% activity of peptide  
 419 at 10 $\mu$ M in the neutrophil assay described below. Those that have  
 30 asterisks exhibit only 30-40% activity of peptide 419 on neutrophils.

In a fifth aspect, the present invention provides a method of treating  
 a subject in order to improve neutrophil and/or monocyte/macrophage  
 function such that infection may be either prevented or treated more  
 effectively, the method comprising administering to the subject a therapeutic  
 35 amount of the peptide of any one of the first to fourth aspects of the present  
 invention.

The peptide may be administered with a pharmaceutically acceptable carrier or adjuvant.

In a preferred embodiment of the fifth aspect of the present invention, the subject is suffering from one or more of the following:

- 5 acquired immunity deficiency syndrome (AIDS); cancer; diabetes; nosocomial infection; tuberculosis; cystic fibrosis; community acquired pneumonia; meningitis; Chlamydia; Legionnaires Disease; Listeriosis; coccidian parasitical (e.g. *Toxoplasma gondii*) infection; an inherited primary neutropenic disorder (e.g. Kostermann's syndrome, mild chronic  
10 neutropaenia and cyclic neutropaenia); an inherited primary defect of phagocytic cell function (e.g. chronic granulomatous disease, myeloperoxidase deficiency, Chediak-Higashi syndrome, specific granule deficiency and Job's syndrome); an inherited secondary defect of phagocytic cell function (e.g. agammaglobulinemia or complement deficiency  
15 syndromes); an acquired defect of phagocytic cell function (e.g. diabetes mellitus, malnutrition, disseminated malignancy, age (elderly or neonates) and alcoholism) immunosuppression due to administration of immunosuppressive drugs; and other bacterial, fungal (e.g. *Candida*), viral or protozoan (e.g. *Pneumocystis carinii*) infection.

- 20 More particularly, where the subject is administered a class 1 peptide, the subject may be suffering from bacterial, fungal, viral (e.g. cytomegalovirus (CMV) infection of neutrophils) or protozoan infection, or cancer and the peptide may be used with myelosuppressive agents to provide an early beneficial enhancement of neutrophil function. Class 1 peptides  
25 may also be beneficial in the treatment of a subject suffering from AIDS since monocyte/macrophage activation is undesirable in order to avoid an increase in viral replication. Other conditions that may be beneficially treated with a class 1 peptide include: cancer; inherited or acquired neutropenic disorders; infectious mononucleosis, paroxysmal nocturnal hemoglobinuria;  
30 conditions where bone marrow infiltration occurs such as in leukemia, lymphoma and myelofibrosis; and inherited primary or secondary defects of phagocytic cell function.

- Where the subject is administered a class 2 peptide, the subject may be suffering from a bacterial infection with, for example, any of  
35 *Pseudomonas*, *E. coli*, *S. aureus* and *S. pneumonia*, a fungal (e.g. *C. albicans* and *T. glabrata*) infection, viral (e.g. CMV) infection, norcardiosis (e.g. *N.*

*asteroides* and *N. brasiliensis*), or protozoan infection. When used in combination with other agents (e.g. antibiotics), class 2 peptides may be particularly useful in preventing or treating chronic lung infection by these organisms (such as *H. influenzae* in COPD and CF), acute community  
5 acquired pneumonia, treatment of skin and soft tissue infections, and treatment of inherited primary or acquired neutropenic disorders and other inherited or acquired defects of phagocytic cell function. Other particular conditions for which class 2 peptides may be useful include: cancer; cystic  
10 fibrosis; Legionnaires disease; tuberculosis; diabetes; meningitis and nosocomial infection.

Finally, where the subject is administered a class 3 peptide, the subject may be at risk of or suffering from a condition caused by intracellular pathogens including Mycobacteria (e.g. *M. leprae*, *M. kansasii*, *M. melonae*,  
15 *M. tuberculosis*, *M. avium* complex, *M. marinum* and *M. ulcerans*), Chlamydia (e.g. *C. pneumoniae*, *C. psittaci* and *C. trachomatis*); Brucellae, Francisella (e.g. *F. tularensis*), Pasteurellosis (e.g. *P. multocida*), Legionellosis (e.g. *L. pneumophila*), Histoplasmosis (e.g. *H. capsulum*) Listeriosis, *Pneumocystis carinii* and *Trypanosoma cruzi*. Another condition that may be beneficially  
20 treated with class 3 peptides is cancer and, particularly, Chronic lymphocytic leukemia where T cell numbers are depressed by chemotherapy with fludarabine and 2-cyclodeoxyadenosine (Girmenia *et al.*, 1994, Brit. J. Haematol. 87: 4078) and macrophage function as measured by TNF $\alpha$  production induced by bacterial products is defective (Dahlke *et al.*, 1995, J. Haematol. 49: 76-82; Fleiger *et al.*, 1990, Int. J. Cancer 45: 280-286). The  
25 peptides having been shown to prevent relapse of mycobacterial infection when CD4 cells are depleted.

The peptides according to the invention may be co-administered (i.e. used in combination therapy) with other therapeutic agents such as cyclosporin A and prednisolone (i.e. for T cell immunosuppressive therapies  
30 for, for example, patients with graft vs host disease) or other agents which stimulate increased white blood cell numbers (e.g. G-CSF, GM-CSF or levamisole); cancer chemotherapy agents such as aminoglutethimide, amsacrine, azacitidine, busulfan; carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, cytarabine HCl, dacarbazine, dactinomycin,  
35 deoxycoformycin, doxorubicin, etoposide, floxuride, fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, lomustine,

mechlorethamine, melphalan, mercaptopurine, mitomycin, mitoxantrone, plicamycin and procarbazine HCl, fludarabine, 2-cyclodeoxyadenosine; antibiotic agents such as amikcan, gentamycin, tobramycin, netilmicin, cefalosporins (such as cephalothin, cefaclor, cefamandole, cefixime, 5 ceftriaxone), penicillins (such as penicillin G), ampicillin, nafcillin, oxacillin, ticascillin, tetracycline, deoxycycline, imipenem, aztreonam, erythromycin, clindamycin, vancomycin, metrimidazole, pyrazinamide, ethambutal, rifabutin, azithromycin, chloritromycin, amikacin, cefoxitin, rifampin and isoniazid; anti-fungal agents such as amphotericin B, ketoconazole and 10 flucytosine; and anti-viral agents such as ganciclovir and acyclovir.

With regard to such combination therapies reference herein to "co-administration" of a peptide according to the invention with other therapeutic agents, is to be understood as including single administration/dosage with a formulation comprising the peptide and 15 therapeutic agent, as well as sequential separate administration/dosage of the peptide and the therapeutic agent. Sequential administration/dosage may be in either order with the administration/dosage separated by, for example, several minutes or up to 24 hours or more.

It will be appreciated by those skilled in the art that a number of 20 modifications may be made to the peptides according to the present invention without deleteriously affecting the biological activity of the peptides. Such modifications include various changes such as sulfation, phosphorylation, nitration, halogenation, and insertions, deletions, and substitutions, either conservative or non-conservative (eg to amino acids, 25 desamino acids) in the peptide sequence where such changes do not substantially alter the overall biological activity of the peptide. By conservative substitutions, the intended combinations are - G, A; V, I, L, M; D, E; N, Q; S, T; K, R, H; F, Y, W, H; and P, N $\alpha$ -alkylamino acids.

It may also be possible to add various groups to the peptides of the 30 present invention to confer advantages such as increased potency or extended half-life *in vivo*, without substantially altering the overall biological activity of the peptide.

The term peptide is to be understood to embrace peptide bond replacements (isosteres) and/or peptide mimetics, ie pseudopeptides, as 35 recognised in the art (see for example: Proceedings of the 20th European Peptide Symposium, ed. G. Jung, E. Bayer, pp. 289-336, and references

therein), as well as salts and pharmaceutical preparations and/or formulations which render the bioactive peptide(s) particularly suitable for oral, topical, nasal spray, ocular pulmonary, IV, subcutaneous, as the case may be, delivery. Such salts, formulations, amino acid replacements and pseudopeptide structures may be necessary and desirable to enhance the stability, formulation, deliverability (eg, slow release, prodrugs), or to improve the economy of production, and they are acceptable, provided they do not negatively affect the required biological activity of the peptide.

Apart from substitutions, three particular forms of peptide mimetic and/or analogue structures of particular relevance when designating bioactive peptides, which have to bind to a receptor while risking the degradation by proteinases and peptidases in the blood, tissues and elsewhere, may be mentioned specifically, illustrated by the following examples: Firstly, the inversion of backbone chiral centres leading to D-amino acid residue structures may, particularly at the N-terminus, lead to enhanced stability for proteolytical degradation while not impairing activity. An example is given in the paper "Tritiated D-ala<sup>1</sup>-Peptide T Binding", Smith, C. S. *et al*, Drug Development Res. 15, pp. 371-379 (1988). Secondly, cyclic structures for stability, such as N to C interchain imides and lactames (Ede *et al* in Smith and Rivier (Eds) "Peptides: Chemistry and Biology", Escom, Leiden (1991), p268-270), and sometimes also receptor binding may be enhanced by forming cyclic analogues. An example of this is given in "Conformationally restricted thymopentin-like compounds", US Patent No. 4,457,489 (1985), Goldstein, G. *et al*. Finally, the introduction of ketomethylene, methylsulfide or retroinverse bonds to replace peptide bonds, ie the interchange of the CO and NH moieties may both greatly enhance stability and potency. An example of the latter type is given in the paper "Biologically active retroinverse analogues of thymopentin", Sisto A. *et al* in Rivier, J. E. and Marshall, G. R. (Eds) "Peptides, Chemistry, Structure and Biology", Escom, Leiden (1990), p. 722-773.

The peptides of the invention can be synthesised by various methods which are known in principle, namely by chemical coupling methods (cf. Wunsch, E.: "Methoden der organischen Chemie", volume 15, Band 1 + 2, Synthesen von Peptiden, Theime Verlag, Stuttgart (1974), and Barrany, G.; Merrifield, R. B: "The Peptides", eds. E. Gross, J. Meienhofer., Volume 2, Chapter 1, pp. 1-284, Academic Press (1980), or by enzymatic coupling

methods (cf. Widmer, F., Johansen, J. T., Carlsberg Res. Commun., volume 44, pp. 37-46 (1979), and Kullman, W.: "Enzymatic Peptide Synthesis", CRC Press Inc., Boca Raton, Florida (1987), and Widmer, F., Johansen, J. T. In "Synthetic Peptides in Biology and Medicine", eds., Alitalo, K., Partanen, P.,  
5 Vatieri, A., pp. 79-86, Elsevier, Amsterdam (1985), or by a combination of chemical and enzymatic methods if this is advantageous for the process design and economy.

It will be seen that one of the alternatives embraced in the general formula set out above is for a cysteine residue to be positioned at both the  
10 amino and carboxyl terminals of the peptide. This will enable the cyclisation of the peptide by the formation of a di-sulphide bond.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated component or feature or group of components or features with or without the  
15 inclusion of a further component or feature or group of components or features.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described by way of the following non-limiting examples and accompanying figures.

20

**Brief description of the accompanying figures:**

Figure 1: Provides graphical results of nitrite release by BCG-infected bone marrow-derived macrophages activated with peptides 966, 927, 926, 419  
25 or 968 and interferon  $\gamma$ .

Figure 2: Provides graphical results showing stimulation of nitrite release by BCG-infected bone marrow-derived macrophages by peptide 1065.

30 Figure 3: Provides graphical results of recrudescence of splenic BCG (disseminated mycobacterial) levels following CD4 cell depletion - effect of peptide treatment.

Figure 4: Provides graphical results of the mean number of liver  
35 granulomas following CD4 depletion in BCG (disseminated mycobacterial) infection.

Figure 5: Provides graphical results showing the effect of peptide 966 on infection-related weight loss in chronic *Pseudomonas aeruginosa* lung infection.

5

Figure 6: Provides graphical results showing the effect of peptide 968 on infection-related weight loss in chronic *Pseudomonas aeruginosa* lung infection.

10 **Production of human TNF peptides tested for neutrophil stimulatory activity:**

The following peptides were synthesised according to well known methods and are described using the I.U.P.A.C. one-letter code abbreviations for amino acid residues.

15

Peptide 419	PSTHVLITHTI
Peptide 757	PSTHVLITHT
Peptide 758	PSTHVLITH
Peptide 759	PSTHVLIT
Peptide 760	PSTHVL
Peptide 771	STHVLITHTI
Peptide 772	THVLITHTI
Peptide 773	HVLITHTI
Peptide 774	VLITHTI
Peptide 775	LITHTI
Peptide 761	THTI
Peptide 776	AcPSTHVLITHTI
Peptide 777	Ac-G-C(Acm)PSTHVLITHTI
Peptide 778	Ac-PSTHVLITHTI-NH <sub>2</sub>
Peptide 923	SSTHVLITHTI
Peptide 925	P-βAla-STHVLITHTI
Peptide 926	βAla-PSTHVLITHTI
Peptide 927	PSTHVIITHTI
Peptide 933	Suc-PSTHVLITHTI
Peptide 934	Nip-STHVLITHTI
Peptide 966	βAla-PSTHVIITHTI



Peptide 967	P- $\beta$ Ala-STHVIIHTI
Peptide 968	Nip-STHVIIHTI
Peptide 973	PSTHVLITHT $\tau$ me-HTI
Peptide 1037	$\beta$ Ala-P- $\tau$ me-STHVIIHTI
Peptide 1059	RSTHVIIHTI
Peptide 1060	KSTHVIIHTI
Peptide 1061	HSTHVIIHTI
Peptide 1062	ASTHVIIHTI
Peptide 1063	LSTHVIIHTI
Peptide 1064	WSTHVIIHTI
Peptide 1065	MSTHVIIHTI
Peptide 1066	NSTHVIIHTI
Peptide 1067	ESTHVIIHTI
Peptide 1068	RRSTHVIIHTI
Peptide 1069	KRSTHVIIHTI
Peptide 1070	HRSTHVIIHTI
Peptide 1071	ARSTHVIIHTI
Peptide 1072	RPSTHVIIHTI
Peptide 1073	KPSTHVIIHTI
Peptide 1074	HPSTHVIIHTI
Peptide 1075	APSTHVIIHTI
Peptide 1076	IPSTHVIIHTI
Peptide 1077	WPSTHVIIHTI
Peptide 1078	MPSTHVIIHTI
Peptide 1079	SPSTHVIIHTI
Peptide 1080	NPSTHVIIHTI
Peptide 1081	EPSTHVIIHTI
Peptide 1083	$\gamma$ AbuPSTHVIIHTI
Peptide 1084	DbuPSTHVIIHTI
Peptide 1085	SarPSTHVIIHTI
Peptide 1086	N-Me-Ala- PSTHVIIHTI
Peptide 1087	RAPSTHVIIHTI
Peptide 1088	AAPSTHVIIHTI
Peptide 1089	GAPSTHVIIHTI
Peptide 1090	EAPSTHVIIHTI

Peptide 1097	ASTHVIITHTI
Peptide 1098	PATHVIITHTI
Peptide 1099	PSAHVIITHTI
Peptide 1100	PSTAVIITHTI
Peptide 1101	PSTHAIITHTI
Peptide 1102	PSTHVAITHTI
Peptide 1103	PSTHVIATHTI
Peptide 1104	PSTHVILAHTI
Peptide 1105	PSTHVIITATI
Peptide 1106	PSTHVIITHAI
Peptide 1107	PSTHVIITHTA
Peptide 1108	PSTHVd-IITHTI
Peptide 1109	PSTHVFITHTI
Peptide 1161	SPSTAVIITHTI
Peptide 1162	SPSTHVLITHTI
Peptide 1163	SPSTHVIITHLI
Peptide 1164	SPSTHVIITHII
Peptide 1165	SPSTHVIITHd-II
Peptide 1166	SPSTHVIITHTd-I
Peptide 1168	MSTAVIITHTI
Peptide 1169	MSTHVLITHTI
Peptide 1170	MSTHVIITHLI
Peptide 1171	MSTHVIITHII
Peptide 1172	MSTHVIITHII
Peptide 1173	MSTHVIITHTd-I
Peptide 1175	NipSTAVIITHTI
Peptide 1176	NipSTHVLITHTI
Peptide 1177	NipSTHVIITHLI
Peptide 1178	NipSTHVIITHII
Peptide 1179	NipSTHVIITHd-II
Peptide 1180	NipSTHVIITHTd-I

Ac represents acetyl, and Acn represents acetamidomethyl.

**EXAMPLE 1****Effect of Peptides on neutrophil function:****Chemiluminescence assay:**

5 Human neutrophils were treated with peptides for 15 minutes according to the protocol described by Ferrante *et al.* 1988 (*Int. Arch. Allergy Appl. Immunol.*, 86; 82-91) and lucigenin-dependent chemiluminescence measured.

10 **Duration of action of Peptides *in vivo*:**

All peptides were administered ip at 4mg/kg. At various times post inoculation peritoneal cells were harvested and chemiluminescence in response to the bacterial peptide fMLP measured in duplicate to quadruplicate determinations. The data represent the mean  $\pm$ SD of 3-15  
15 animals.

The peritoneal cells were harvested by the following method: Sterile, pyrogen-free Hanks Balanced Salt Solution (5ml) was injected intraperitoneally following exposure of the peritoneal cavity. The contents were massaged for 30 seconds and the peritoneal fluid carefully withdrawn.  
20 Cells contained in the peritoneal fluid were washed, counted by trypan blue exclusion (>99% viability) and resuspended to  $10^6$  macrophages/ml.

Peptides 757-761, 771-778 and 1097-1107 were synthesised and tested for neutrophil stimulation in order to determine the "core" residues and  
25 sequence of peptide 419 required for neutrophil stimulatory activity. As can be seen from Table 1, N and C-terminal truncations of peptide 419 significantly reduced activity, with only the truncated peptides 771 (STHVLITHTI), 757 (PSTHVLITHT) and 758 (PSTHVLITH) retaining approximately 40% or more of the activity of peptide 419. Consequently, it is  
30 predicted that the core sequence STHVLITH would exhibit approximately 40% of the activity of peptide 419. Within this core sequence, it is apparent that the position 1 (Ser), position 2 (Thr), position 4 (Val), position 6 (Ile), position 7 (Thr) and position 8 (His) are essential for retaining significant activity (see Table 2).

**TABLE 1:      ACTIVITY OF TRUNCATED FORMS OF PEPTIDE 419 IN**  
**NEUTROPHIL CHEMILUMINESCENCE ASSAY**

<b>Peptide</b>	<b>Activity at 10 <math>\mu</math>M compared to 419</b>
419	100
771	39.3
772	9.3
773	20
774	7.8
775	8.0
761	0
776	57.9
777	45.7
778	25
757	56.9
758	50
759	15
760	15

**TABLE 2:      CORE SEQUENCES OF PEPTIDE 419**

<b>Peptide</b>	<b>Neutrophil stimulatory activity relative to 419 (%)</b>
1062	100
1098	7.7
1099	3.2
1100	128
1101	2.8
1102	2.7
1103	8.8
1104	7.9
1105	51.0
1106	13.1
1107	10.9

With knowledge of the above core residues and sequences, modifications of peptide 419 were designed and synthesised. These peptides show similar activities to peptide 419 *in vitro* (Tables 2 to 9), however some  
5 (e.g. peptide 966 and 968) show a duration of action and level of cellular activation which is significantly enhanced (Tables 10 and 11). This effect would be expected to lead to enhanced antimicrobial activity as indicated by increased resistance to infection and/or enhanced clearance of invading  
10 organisms. In addition, less frequent administration and/or more favourable delivery of the peptide, e.g. via oral absorption, would be expected as a consequence of the increased stability and longer duration of action of the novel peptides.

**TABLE 3: STIMULATION OF HUMAN NEUTROPHILS BY PEPTIDES**

Treatment	Peak chemiluminescence (mV)				
	Expt 1	2	3	4	5
Control	130	123	15	64	40
419 10 $\mu$ M	454	628	98	480	552
1	145	237	25	204	211
0.1	125	174	19	ND	ND
923 10 $\mu$ M	623	ND	ND	ND	ND
1	307	ND	ND	ND	ND
0.1	148	ND	ND	ND	ND
925 10 $\mu$ M	538	ND	ND	351	445
1	289	ND	ND	143	203
0.1	151	ND	ND	ND	ND
926 10 $\mu$ M	423	ND	ND	414	582
1	233	ND	ND	175	274
0.1	ND	ND	ND	ND	ND
927 10 $\mu$ M	603	ND	ND	498	ND
1	360	ND	ND	259	ND
0.1	ND	ND	ND	ND	ND
934 10 $\mu$ M	ND	478	ND	ND	ND
1	ND	400	ND	ND	ND
0.1	ND	193	ND	ND	ND
966 10 $\mu$ M	ND	ND	ND	382	415
1	ND	ND	ND	195	272
0.1	ND	ND	ND	ND	ND
967 10 $\mu$ M	ND	ND	ND	428	477
1	ND	ND	ND	181	239
0.1	ND	ND	ND	ND	ND
968 10 $\mu$ M	ND	ND	98	ND	ND
1	ND	ND	34	ND	ND
0.1	ND	ND	15	ND	ND

**TABLE 4:      STIMULATION OF HUMAN NEUTROPHILS BY PEPTIDES**

Treatment	Peak Chemiluminescence (mV)	
	Expt No.	
	6	7
HBSS	30.4±9.6	43.97±6.3
419	248±51.2	222±23.4
1059	319±27.9	223.3±9.3
1060	225±8.9	176.7±56.9
1061	247±37.9	279.3±67.7
1063	275±67.5	385.3±44.1
1064	172±15.1	165.7±14.0
1065	614±52.3	594.3±28.6
1066	291±65.2	202±50.2
1067	251.5±110.7	234±46.7
1068	141.6±29.7	168±24.6
1069	140±23.9	138±9.5
1070	165±32.2	265±2.1
1071	151.5±48.8	194±4.4
1072	130±41.2	196±31.6
1073	150±45.8	165±18.9
1074	303±86.2	162±79.8

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.

TABLE 5: STIMULATION OF HUMAN NEUTROPHILS BY PEPTIDES

Treatment	Peak Chemiluminescence (mV) Expt No. 8
HBSS	30.1±3.7
419	208±63.9
966	159±9.0
1075	189±24.6
1076	204±49.6
1077	133±9.0
1078	203±26.1
1079	178±31.2
1080	150±9.8
1081	135±34.3
1083	168±23.6
1084	129±33.3
1085	157±31.8
1086	171±21.7
1087	148±42
1088	66.8±20
1089	136.9±39.3
1090	90.9±20.5

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.



**TABLE 6:      STIMULATION OF HUMAN NEUTROPHILS BY PEPTIDES**

Treatment	Peak Chemiluminescence (mV) "
	Expt No. 9
HBSS	10.56±3.07
Control Peptide	7.44±3.15
419	192.7±75.4
927	69.9±34.7
1062	46.97±7.85
1098	24.6±4.08
1099	16.4±2.65
1101	15.7±3.67
1102	15.5±3.46
1103	26.6±4.69
1104	24.9±6.24
1106	34.4±11.5
1107	30.4±3.78
1108	6.97±2.08
1109	35.9±18.8

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.

**TABLE 7:        STIMULATION OF HUMAN NEUTROPHILS BY PEPTIDES**  
**419, 1037, 966, 927, 1100, 1105**

Treatment	Peak Chemiluminescence (mV)
	Expt no. 10
HBSS	60.8±2.76
Control Peptide	62.9±12.4
419	188.3±15.3
1037	157.3±23.9
966	143.3±40.0
927	167.7±25.6
1100	224.5±9.19
1105	126±28.2

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.

**TABLE 8:        EFFECT OF PEPTIDES ON NEUTROPHIL ACTIVATION**

Treatment	Peak Chemiluminescence (mV)
	Expt No. 11
HBSS	5.02±1.58
419	192.7±75.4
1108	6.97±2.08
1109	35.9±18.8

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.

TABLE 9: STIMULATION OF HUMAN NEUTROPHILS BY PEPTIDES

Treatment	Peak Chemiluminescence (mV)	
	Expt No.	
	12	13
HBSS	36.9±3.2	58.5±1.04
419	267.7±53.8	482.3±16.5
1161	199.3±20.6	
1162	162±13.5	
1163	75.1±17.8	
1164	171.7±20.4	
1165	146±10.4	
1166	156±19.9	
1168	114.6±19.1	
1169	272.7±6.03	
1170		278±5.69
1171		386.7±8.74
1172		304.3±18.1
1173		279.7±22.2
1175		315±77.7
1176		245±12.4
1177		218±13.5
1178		289±14.7
1179		183±15.9
1180		175±28.7

**TABLE 10: EX VIVO CHEMILUMINESCENCE RESPONSE OF  
MURINE PERITONEAL CELLS FOLLOWING  
INTRAPERITONEAL INOCULATION OF PEPTIDE**

Treatment	Response to fMLP peak chemiluminescence (mV)			
	Hours post inoculation			
	1	2	5	18
Control	2.3±0.91 (15)	1.92±0.83 (6)	2.47±0.40 (6)	4.15±1.2 (3)
419	2.14±0.59 (11)	4.99±1.24 (6)	4.90±1.43 (6)	3.67±2.84 (11)
923	1.48±0 (2)	3.43±0.80 (3)	2.04±0.32 (5)	4.65±0.36 (3)
925	1.75±0.36 (6)	20.2±3.67 (6)	10.45±5.68 (6)	2.14±0.47 (6)
926	2.95±0.30 (3)	6.28±0.61 (3)	2.43±-.19 (3)	1.55±0.12 (3)
933	2.36±0.56 (6)	4.4±1.16 (6)	8.25±1.37 (6)	6.31±1.88 (6)
966	3.09±0.2 (3)	9.64±2.88 (9)	19.18±6.98 (9)	9.64±5.72 (9)
967	2.05±0.006 (3)	4.30±0.23 (3)	16.48±0.55 (3)	4.10±0.73 (3)
968	2.40±0.04 (3)	9.95±0.01 (3)	17.58±3.11 (6)	5.86±0.52 (6)
1065	-	-	9.71±1.39 (3)	-
1100	-	-	23.2±3.9 (3)	5.73±0.06 (3)

All peptide treated animals received 4mg/kg peptide.

Control animals were injected with sterile, pyrogen free phosphate-buffered saline.

Results are expressed as the mean±standard deviation of determinations from 3-15 animals.

The number of animals used is indicated in brackets.

TABLE 11

Treatment Peptide	Basal response Peak chemiluminescence (mV) Hours post inoculation			
	1	2	3	4
923	1.48±0.07 (3)	2.88±0.25 (3)	1.99±0.27 (3)	4.29±0.54 (3)
925	2.06±0.75 (6)	8.49±1.18 (6)	4.66±0.80 (6)	2.12±0.59 (6)
926	3.02±0.41 (3)	3.74±0.19 (3)	1.50±0.06 (3)	1.70±0.06 (3)
933	1.86±0.23 (6)	2.64±0.89 (6)	6.04±1.07 (6)	25.23±0.94 (6)
966	2.79±0.26 (3)	5.24±1.07 (9)	9.19±4.01 (9)	5.47±1.31 (9)
967	1.86±0.06 (3)	2.17±0.19 (3)	4.37±0.53 (3)	3.26±0.32 (3)
968	3.13±0.17 (3)	6.47±0.55 (3)	9.28±4.25 (6)	4.90±0.70 (6)
1065	-	-	3.75±0.44 (3)	-
1100	-	-	9.27±0.94 (3)	3.70±0.4 (3)

## EXAMPLE 2

**Peptides and evaluation for neutrophil and monocyte/macrophage stimulatory activity:**

- 5           Peptides 419, 966, 968 and 1059-1081, 1083-1090 were assessed for neutrophil and monocyte/macrophage stimulatory activity. Results are presented in Tables 12 to 14. Neutrophil stimulatory activity was determined by the chemiluminescence assay described in Example 1.
- 10          Monocyte/macrophage stimulatory activity was determined according to the method described below.

Monocyte chemiluminescence assay:

Monocytes were purified from the blood of normal volunteers by centrifugation in Ficoll-Hypaque medium followed by overnight adherence to cytodex microcarriers (Kumaratilake and Ferrante, 1988, *J. Immunol Methods* 5 112: 183-190). Monocyte stimulation by peptides was measured by lucigenin-dependent chemiluminescence as described for neutrophils.

**TABLE 12:** **PEPTIDES EVALUATED FOR NEUTROPHIL AND MONOCYTE/MACROPHAGE STIMULATORY ACTIVITY**

Peptide	Ratio monocyte/neutrophil activity at 10 $\mu$ M
419	1
1059	1.57
1060	1.25
1061	0.80
1062	1.00
1063	6.65
1064	0.63
1065	12.60
1066	1.88
1067	6.4
1068	2.84
1069	1.08
1070	2.10
1071	2.11
1072	3.66
1073*	-
1074	2.10
1075*	-
1076	3.40
1077	5.85
1078	12.80
1079	0.05
1080	1.25
1081	2.03
966*	0.71
1083	1.77
1084	3.25
1085	2.07
1086	2.42
1087	4.04
1088	5.30
1089	3.20
1090	6.25
968	1.38

These peptides did not activate monocytes, i.e. appeared to be neutrophil specific.

**TABLE 13: STIMULATION OF HUMAN MONOCYTES BY PEPTIDES**

Treatment	Peak Chemiluminescence (mV)				
	Expt No.				
	1	2	3	4	5
HBSS	3.65±0.2	5.49±0.98	12.72±3.94	2.35±0.04	1.94±0.22
419	5.25±0.9	7.89±0.93	14.8±1.11	4.14±0.28	2.82±0.08
1059	6.88±0.77	-	-	-	-
1060	5.45±0.99	-	-	-	-
1061	4.45±0.32	-	-	-	-
1062	5.18±0.34	-	-	-	-
1063	-	23.2±1.59	-	-	-
1064	-	6.54±0.36	-	-	-
1065	-	80.4±6.51	-	-	-
1066	-	10.89±2.15	-	-	-
1067	-	21.07±2.17	-	-	-
1068	-	9.44±0.92	-	-	-
1069	-	6.95±1.32	-	-	-
1070	-	8.85±0.98	-	-	-
1071	-	8.61±0.69	-	-	-
1072	-	10.1±1.08	-	-	-
1073	-	-	12.6±1.18	-	-
1074	-	-	18.07±2.06	-	-
1075	-	-	12.1±1.1	-	-
1076	-	-	19.8±2.59	-	-
1077	-	-	20.5±1.26	-	-
1078	-	-	38.7±2.78	-	-
1079	-	-	-	2.82±0.11	-
1080	-	-	-	3.61±1.12	-
1081	-	-	-	4.0±1.06	-
966	-	-	-	2.94±0.09	-
1083	-	-	-	4.09±1.02	-
1084	-	-	-	4.64±1.15	-
1085	-	-	-	-	3.31±0.63
1086	-	-	-	-	3.69±0.53
1087	-	-	-	-	4.47±0.85
1088	-	-	-	-	3.44±0.46
1089	-	-	-	-	3.78±1.35
1090	-	-	-	-	4.34±0.95

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.



**TABLE 14:      EFFECT OF PEPTIDES ON NEUTROPHIL AND  
MONOCYTE ACTIVATION**

Treatment	Peak Chemiluminescence (mV)	
	Neutrophil	Monocyte
HBSS	34.66±4.56	20.8±8.8
419	124.3±40.5	61.0±4.6
973	45.7±16.6	23±9.3

All peptides were at 10µM final concentration.

Values represent the mean±SD of triplicate determinants.

### EXAMPLE 3

5      **Peptides tested for effect on nitrite release by *M. bovis* BCG-infected bone  
marrow macrophage (BMM):**

Peptides 419, 926, 927, 966, 968 and 1065 were assessed for their effect on nitrite release by *M. bovis* BCG-infected bone marrow macrophage.

10      **Measurement of macrophage nitric oxide production and inhibition of BCG  
growth by peptides:**

15      Mouse macrophages were derived from the bone marrow following isolation of progenitor cells and subsequent growth for 6 days in RMI-1640 containing 5% horse serum, 10% foetal calf serum, 20% L929 cell supernatant, 2mM L-glutamine, 2-mercaptoethanol, streptomycin and penicillin. Peptides (0.6-60µM final) were added to the macrophage cultures (10<sup>5</sup> macrophages/well) 24 hours prior to infection with 10<sup>6</sup> *Bacillus Cametre* - Guerin (BCG) organisms. Three days after infection the degree of growth inhibition was determined by a 24 hour pulse of <sup>3</sup>H-Uracil (1.0µCi/well). Nitric oxide content of the cell supernatant was also measured three days after infection by reaction with the Greiss reagent and optical density of the reaction at 540nm determined.

20

Results are provided in Figures 1 and 2.

**EXAMPLE 4****Peptides tested for effect on recrudescence of infections shown by splenic BCG levels and granulomatous pathology following CD4 cell depletion:**

5           Peptides 419, 966 and 968 were tested for their effect on  
necrudescence of BCG infection *in vivo* following CD4 depletion.

          Mice were infected with  $1 \times 10^6$  BCG iv. Thirteen weeks after  
infection when the infection had become chronic, mice were treated (300µg  
Mab daily for three days and then at weekly intervals for 4 weeks) with  
10       monoclonal antibody GKI-54 which binds to CD4<sup>+</sup> lymphocytes. All mice  
received ip injection of saline or 100µg of peptide every second day for 4  
weeks. Mice were then sacrificed and the number of splenic BCG organisms  
in each was determined by growth on 7HII agar plates (Figure 3). The  
number of granulomas in liver, as a measure of immunopathology, was  
15       determined by staining of frozen sections with the P7/9 Mab which  
recognises murine MHC class 11. Quantification of staining was carried out  
using Image Analysis (Chromatic colour image analysis software package  
version 2.2 from Leading Edge) (Figure 4).

20

**EXAMPLE 5****Effect of peptides 966 and 968 on *Pseudomonas aeruginosa* lung infection:**

*Pseudomonas aeruginosa* (stain #16800) was grown overnight on  
25       blood agar plates from frozen stock. The organisms were encapsulated in  
agarose (Calbiochem 2% w/v in sterile phosphate buffered saline). Mice were  
infected intracheally with 30µl of agarose beads containing 6,000-12,000  
CFU. Peptide treatments were given at t=-24 and t=-2 hours relative to  
infection and then daily during the course of the study. On day 5 post  
30       infection mice were treated two hours prior to sacrifice for enumeration of  
lung bacteria. The lungs were excised and homogenised. Serial dilutions of  
the lung homogenate were then plated onto blood agar and incubated  
overnight at 37°C. The number of colonies in each plate was then counted.  
Infection-related weight loss was monitored during the course of the  
35       infection by daily weighing of all animals (Figures 5 and 6, and Table 15).

TABLE 15

Treatment		CFU at Sacrifice
Saline	1	$2.18 \times 10^6$
	2	$6.06 \times 10^6$
968 400 $\mu$ g/kg	1	$1.00 \times 10^3$
	2	$3.30 \times 10^4$
	3	$1.80 \times 10^3$
	4	$5.00 \times 10^3$
968 40 $\mu$ g/kg	1	$2.02 \times 10^4$
	2	$1.98 \times 10^4$
	3	$1.00 \times 10^3$
	4	$6.00 \times 10^2$

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

**CLAIMS:**

1. A peptide with neutrophil and/or monocyte/macrophage stimulatory activity, wherein the peptide is of the general formula:-

5  $X_1-X_2-X_3-X_4$ -Ser-Thr- $X_5$ -Val- $X_6$ -Ile-Thr- $X_7$ - $X_8$ - $X_9$ - $X_{10}$

in which,

$X_1$  is absent, Cys or  $R_1$ ,

$X_2$  is absent, Ala, Arg, Glu or Gly,

10  $X_3$  is absent, Ala, Arg, Asn, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Trp,  $\gamma$ -Abu,  $\beta$ Ala, Dbu, Sar, Suc or N-Me-Ala,

$X_4$  is absent, Ala, Arg, Asn, Glu, His, Leu, Lys, Met, Pro, Ser, Trp,  $\beta$ Ala or Nip,

$X_5$  is Ala or His,

15  $X_6$  is Ala, Gly, Ile, Leu, Phe, Pro, Ser, Thr, Trp, Val, D-Ala, D-Ile D-Pro, D-Ser, D-Thr, D-Val or  $\beta$ Ala,

$X_7$  is His or Ala,

$X_8$  is absent, Ile, Leu, Thr or D-Ile,

$X_9$  is absent, Ile, D-Ile or Aib, and

$X_{10}$  is absent, Cys or  $R_2$ ,

20  $R_1$  is H or R-CO, where R is H, straight, branched or cyclic alkyl up to C20, optionally containing double bonds and/or substituted with halogen, nitro, amino, hydroxy, sulfo, phospho or carboxyl groups which may be substituted themselves or aralkyl or aryl optionally substituted as listed for the alkyl or  $R_1$  is glycosyl, nucleosyl or lipoyl and  $R_1$  is absent when the amino acid adjacent is an unsubstituted desamino-derivative;  $R_2$  is

25 -NR<sub>12</sub>R<sub>13</sub>, wherein R<sub>12</sub> and R<sub>13</sub> are independently H, straight, branched or cyclic alkyl, aralkyl or aryl optionally substituted as defined for  $R_1$  or  $R_2$  is N-glycosyl or N-lipoyl, or  $R_2$  is -OR<sub>14</sub>, where R<sub>14</sub> is H straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for  $R_1$  or  $R_2$  is  
30 -O-glycosyl, or -O-lipoyl or  $R_2$  is absent when the adjacent amino acid is a dicarboxy derivative of cysteine or a homologue thereof or the peptide is in a N-C cyclic form; with the proviso that the peptide is not Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile.

35 2. A peptide according to claim 1, wherein  $X_6$  is Ile.

3. A peptide according to claim 1 or 2, wherein  $X_1$  and  $X_{10}$  are absent.
4. A peptide according to claim 1 selected from the group consisting of:-
  - 5 Ser-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
Pro- $\beta$ Ala-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 $\beta$ Ala-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Suc-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
10 Nip-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 $\beta$ Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Pro- $\beta$ Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
15 Lys-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
His-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Leu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Trp-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
20 Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Asn-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Glu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Arg-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Lys-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
25 His-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Ala-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Arg-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Lys-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
His-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
30 Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Leu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Trp-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Met-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
35 Asn-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
Glu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,

5       $\gamma$ Abu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Dbu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Sar-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       N-methyl Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Arg-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Ala-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Gly-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Glu-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 10     Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Pro-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Pro-Ser-Thr-His-Val-Ile-Ile-Thr-Ala-Thr-Ile,  
       Pro-Ser-Thr-His-Val-D-Ile-Ile-Thr-His-Thr-Ile,  
       Pro-Ser-Thr-His-Val-Phe-Ile-Thr-His-Thr-Ile,  
       Ser-Pro-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 15     Ser-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
       Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
       Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
       Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile,  
       Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile,  
 20     Met-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Met-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
       Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
       Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
       Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-D-Ile,  
 25     Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile,  
       Nip-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
       Nip-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
       Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
       Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 30     Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and  
       Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

5. A peptide with neutrophil stimulatory activity, wherein the peptide is of the general formula:-

35       $X_1-X_2-X_3-X_4$ -Ser-Thr- $X_5$ -Val- $X_6$ -Ile-Thr- $X_7$ - $X_8$ - $X_9$ - $X_{10}$

in which,

- X<sub>1</sub> is absent, Cys or R<sub>1</sub>,  
 X<sub>2</sub> is absent,  
 X<sub>3</sub> is Ala, Lys or Ser,  
 X<sub>4</sub> is Pro,  
 5 X<sub>5</sub> is Ala or His,  
 X<sub>6</sub> is Ile or Leu,  
 X<sub>7</sub> is Ala or His,  
 X<sub>8</sub> is Ile, Leu, Thr or D-Ile,  
 X<sub>9</sub> is Ile or D-Ile,  
 10 X<sub>10</sub> is absent, Cys or R<sub>2</sub>,

wherein R<sub>1</sub> is H or R-CO, where R is H, straight, branched or cyclic alkyl up to C20, optionally containing double bonds and/or substituted with halogen, nitro, amino, hydroxy, sulfo, phospho or carboxyl groups which may be substituted themselves or aralkyl or aryl optionally substituted as listed for the alkyl or R<sub>1</sub> is glycosyl, nucleosyl or lipoyl and R<sub>1</sub> is absent when the amino acid adjacent is an unsubstituted desamino-derivative; R<sub>2</sub> is -NR<sub>12</sub>R<sub>13</sub>, wherein R<sub>12</sub> and R<sub>13</sub> are independently H, straight, branched or cyclic alkyl, aralkyl or aryl optionally substituted as defined for R<sub>1</sub> or R<sub>2</sub> is N-glycosyl or N-lipoyl, or R<sub>2</sub> is -OR<sub>14</sub>, where R<sub>14</sub> is H straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for R<sub>1</sub> or R<sub>2</sub> is -O-glycosyl, or -O-lipoyl or R<sub>2</sub> is absent when the adjacent amino acid is a dicarboxy derivative of cysteine or a homologue thereof or the peptide is in a N-C cyclic form.

- 25 6. A peptide according to claim 5, wherein X<sub>1</sub> and X<sub>10</sub> are absent.
7. A peptide according to claim 5 selected from the group consisting of:-
- 30 Lys-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Ser-Pro-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Ser-Pro-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 35 Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and

Ser-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

8. A peptide with neutrophil and monocyte/macrophage stimulatory activity wherein the peptide is of the general formula:-

5  $X_1-X_2-X_3-X_4$ -Ser-Thr- $X_5$ -Val- $X_6$ -Ile-Thr- $X_7-X_8-X_9-X_{10}$

in which,

$X_1$  is absent, Cys or  $R_1$ ,

$X_2$  is absent,

$X_3$  is absent, Asn, Lys or  $\beta$ Ala,

10  $X_4$  is Arg, His, Lys, Pro, Trp, Ala or Nip,

$X_5$  is Ala or His,

$X_6$  is Ile or Leu,

$X_7$  is Ala or His,

$X_8$  is Ile, Leu, Thr or D-Ile,

15  $X_9$  is Ile or D-Ile,

$X_{10}$  is absent, Cys or  $R_2$ ,

wherein  $R_1$  is H or R-CO, where R is H, straight, branched or cyclic alkyl up to C20, optionally containing double bonds and/or substituted with halogen, nitro, amino, hydroxy, sulfo, phospho or carboxyl groups which may be substituted themselves or aralkyl or aryl optionally substituted as listed for the alkyl or  $R_1$  is glycosyl, nucleosyl or lipoyl and  $R_1$  is absent when the amino acid adjacent is an unsubstituted desamino-derivative;  $R_2$  is

20 -NR<sub>12</sub>R<sub>13</sub>, wherein R<sub>12</sub> and R<sub>13</sub> are independently H, straight, branched or cyclic alkyl, aralkyl or aryl optionally substituted as defined for  $R_1$  or  $R_2$  is N-glycosyl or N-lipoyl, or  $R_2$  is -OR<sub>14</sub>, where R<sub>14</sub> is H straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for  $R_1$  or  $R_2$  is -O-glycosyl, or -O-lipoyl or  $R_2$  is absent when the adjacent amino acid is a dicarboxy derivative of cysteine or a homologue thereof or the peptide is in a N-C cyclic form; with the proviso that when  $X_3$  is Lys, then  $X_4$  is not Pro.

30

9. A peptide according to claim 8, wherein  $X_1$  and  $X_{10}$  are absent.

10. A peptide according to claim 8 selected from the group consisting of:-

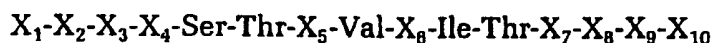
35  $\beta$ Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,

Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,



Lys-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 His-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Ala-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Trp-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 5 Lys-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Asn-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Nip-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Nip-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,  
 10 Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
 Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-D-Ile-Ile, and  
 Nip-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

11. A peptide with monocyte/macrophage stimulatory activity, wherein  
 15 the peptide is of the general formula:-



in which,

X<sub>1</sub> is absent, Cys or R<sub>1</sub>,  
 X<sub>2</sub> is absent, Ala, Arg, Glu or Gly,  
 20 X<sub>3</sub> is absent, Ala, Arg, Glu, His, Leu, Met, Trp,  $\gamma$ -Abu, Dbu, Sar or  
 N-methyl Ala,  
 X<sub>4</sub> is Arg, Asn, Glu, Leu, Met or Pro,  
 X<sub>5</sub> is Ala or His,  
 X<sub>6</sub> is Ile or Leu,  
 25 X<sub>7</sub> is Ala or His,  
 X<sub>8</sub> is Ile, Leu, Thr or D-Ile,  
 X<sub>9</sub> is Ile or D-Ile,  
 X<sub>10</sub> is absent, Cys or R<sub>2</sub>,

wherein R<sub>1</sub> is H or R-CO, where R is H, straight, branched or cyclic alkyl up  
 30 to C<sub>20</sub>, optionally containing double bonds and/or substituted with halogen,  
 nitro, amino, hydroxy, sulfo, phospho or carboxyl groups which may be  
 substituted themselves or aralkyl or aryl optionally substituted as listed for  
 the alkyl or R<sub>1</sub> is glycosyl, nucleosyl or lipoyl and R<sub>1</sub> is absent when the  
 amino acid adjacent is an unsubstituted desamino-derivative; R<sub>2</sub> is  
 35 -NR<sub>12</sub>R<sub>13</sub>, wherein R<sub>12</sub> and R<sub>13</sub> are independently H, straight, branched or  
 cyclic alkyl, aralkyl or aryl optionally substituted as defined for R<sub>1</sub> or R<sub>2</sub> is N-

- glycosyl or N-lipoyl, or  $R_2$  is -OR<sub>14</sub>, where R<sub>14</sub> is H straight, branched or cyclic alkyl, aralkyl or aryl, optionally substituted as defined for  $R_1$  or  $R_2$  is -O-glycosyl, or -O-lipoyl or  $R_2$  is absent when the adjacent amino acid is a dicarboxy derivative of cysteine or a homologue thereof or the peptide is in a N-C cyclic form; with the proviso that when  $X_2$  is absent and  $X_3$  is Ala, then  $X_4$  is not Pro.

12. A peptide according to claim 11, wherein  $X_1$  and  $X_{10}$  are absent.
- 10 13. A peptide according to claim 11 selected from the group consisting of:-

- Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Leu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 15 Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Asn-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Glu-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Arg-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 His-Arg-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 20 Arg-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 His-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Leu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Trp-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Met-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 25 Glu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 $\gamma$ Abu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Dbu-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Sar-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 N-methyl Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 30 Arg-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Ala-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Gly-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Glu-Ala-Pro-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-Ile,  
 Met-Ser-Thr-Ala-Val-Ile-Ile-Thr-His-Thr-Ile,  
 35 Met-Ser-Thr-His-Val-Leu-Ile-Thr-His-Thr-Ile,  
 Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Leu-Ile,

Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-Ile,  
Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Ile-D-Ile, and  
Met-Ser-Thr-His-Val-Ile-Ile-Thr-His-Thr-D-Ile.

- 5 14. A method of treating a subject in order to improve neutrophil and/or monocyte/macrophage function such that infection may be either prevented or treated more effectively, the method comprising administering to the subject a therapeutic amount of a peptide according to any one of the preceding claims.
- 10 15. A method according to claim 14, wherein the subject is suffering from bacterial, fungal, viral or protozoan infection.
- 15 16. A method according to claim 14 or 15, wherein the peptide is co-administered with an antimicrobial or antiviral agent.
- 20 17. A method according to claim 16, wherein the antimicrobial or antiviral agent is selected from the group consisting of: amikcan, gentamycin, tobramycin, netilmicin, cephalosporins, penicillins, ampicillin, nafcillin, oxacillin, ticascillin, tetracycline, deoxycycline, imipenem, aztreonam, erythromycin, clindamycin, vancomycin, metrimidazole, rifampin and isonazid, amphotericin B, ketoconazole, flucytosine, gangcilocvir and acyclovir.
- 25 18. A method according to claim 14, wherein the subject is suffering from graft vs host disease or chronic lymphocytic leukemia.
- 30 19. A method according to claim 18, wherein the peptide is co-administered in combination with cyclosporin A, prednisolone, fludarabine, 2-cyclodeoxyadenosine or other immune suppressant.
20. A method according to claim 14, wherein the subject is suffering from cancer.
- 35 21. A method according to claim 20, wherein the peptide is co-administered with a cancer chemotherapy agent.

22. A method according to claim 21, wherein the cancer chemotherapy agent is selected from the group consisting of: aminoglutethimide, amsacrine, azacitidine, busulfan; carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, cytarabine HCl, dacarbazine, dactinomycin, deoxycoformycin, doxorubicin, etoposide, floxuride, fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, lomustine, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitoxantrone, plicamycin and procarbazine HCl, fludarabine and 2-cyclodeoxyadenosine.
23. A method according to claim 14, wherein the peptide is co-administered with G-CSF, GM-CSF, levamisole or other agent which stimulates increased white blood cell numbers.
24. A method according to any one of claims 14 to 23, wherein the subject is suffering from an inherited primary neutropenic disorder.
25. A method according to any one of claims 14 to 23, wherein the subject is suffering from an inherited primary or secondary defect of phagocytic cell function.
26. A method according to any one of claims 14 to 23, wherein the subject is suffering from an acquired defect of phagocytic cell function.
27. A method according to claim 26, wherein the subject is elderly, neonate, alcoholic or malnourished.
28. A method according to claim 14, wherein the subject is suffering from one or more of the following: acquired immunity deficiency syndrome (AIDS); cancer; diabetes; nosocomial infection; tuberculosis; mycobacterium avium complex, cystic fibrosis; community acquired pneumonia; meningitis; Chlamydia; Legionnaires Disease; Listeriosis; and coccidian parasitical infection.
29. A method according to any one of claims 14 to 28, wherein the peptide is co-administered with interferon gamma.

30. A method of treating a subject in order to improve neutrophil function, the method comprising administering to the subject a therapeutic amount of a peptide according to any one of claims 5 to 7.
- 5 31. A method according to claim 30, wherein the subject is suffering from bacterial, fungal, viral or protozoan infection.
32. A method according to claim 30, wherein the subject is suffering  
10 from cancer.
33. A method according to claim 32, wherein the peptide is administered with a cancer chemotherapy agent.
- 15 34. A method according to claim 33, wherein the cancer chemotherapy agent is selected from the group consisting of: aminoglutethimide, amsacrine, azacitidine, busulfan; carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, cytarabine HCl, dacarbazine, dactinomycin, deoxycoformycin, doxorubicin, etoposide, floxuride, fluorouracil,  
20 hexamethylmelamine, hydroxyurea, ifosfamide, lomustine, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitoxantrone, plicamycin procarbazine HCl, fludarabine and 2-cytodeoxyadenosine.
35. A method according to claim 30, wherein the peptide is co-  
25 administered with G-CSF, GM-CSF, levamisole or other agents which stimulate increased white blood cell numbers.
36. A method according to claim 30 or 35, wherein the subject is suffering from: acquired immunity deficiency syndrome (AIDS); or inherited  
30 or acquired neutropenic disorder.
37. A method according to claim 30, wherein the subject is suffering from one or more of the following: infectious mononucleosis, paroxysmal nocturnal, hemoglobinuria, leukemia, lymphoma, myelofibrosis, inherited  
35 primary or secondary defect of phagocytic cell function, and CMV.

38. A method according to claim 30 or 31, where the peptide co-administered with an antimicrobial or antiviral agent.
39. A method according to claim 38, wherein the antimicrobial or  
5 antiviral agent is selected from the group consisting of: amikcan, gentamycin, tobramycin, netilmicin, cephalosporins, penicillins, ampicillin, nafcillin, oxacillin, ticascillin, tetracycline, deoxycycline, imipenem, aztreonam, erythromycin, clindamycin, vancomycin, metrimidazole, rifampin and  
10 isonazid, amphotericin B, ketoconazole, flucytosine, gangciclovir and acyclovir.
40. A method of treating a subject in whom enhanced neutrophil and/or monocyte/macrophage function is desirable, the method comprising  
15 administering to the subject a therapeutic amount of a peptide according to any one of claims 8 to 10.
41. A method according to claim 40 wherein the subject is suffering from bacterial, fungal, viral or protozoan infection.
- 20 42. A method according to claim 40 or 41, wherein the peptide is co-administered with an antimicrobial or antiviral agent.
43. A method according to claim 42, wherein the antimicrobial or  
25 antiviral agent is selected from the group consisting of: amikcan, gentamycin, tobramycin, netilmicin, cephalosporins, penicillins, ampicillin, nafcillin, oxacillin, ticascillin, tetracycline, deoxycycline, imipenem, aztreonam, erythromycin, clindamycin, vancomycin, metrimidazole, rifampin, isonazid, amphotericin B, ketoconazole, flucytosine, gangciclovir and acyclovir.
- 30 44. A method according to claim 40, wherein the subject is suffering from cancer.
45. A method according to claim 44, wherein the peptide is co-administered with a cancer chemotherapy agent.
- 35

46. A method according to claim 45, wherein the cancer chemotherapy agent is selected from the group consisting of: aminoglutethimide, amsacrine, azacitidine, busulfan; carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, cytarabine HCl, dacarbazine, dactinomycin, 5 deoxycoformycin, doxorubicin, etoposide, floxuride, fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, lomustine, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitoxantrone, plicamycin and procarbazine HCl, fludarabine and 2-cytodeoxyadenosine.
- 10 47. A method according to claim 40, wherein the peptide is co-administered with G-CSF, GM-CSF, levamisole or other agents which stimulate increased white blood cell numbers.
- 15 48. A method according to claim 40 or 47, wherein the subject is suffering from one or more of the following: cancer; cystic fibrosis; community-acquired pneumonia; Legionnaires disease; tuberculosis; diabetes and meningitis.
- 20 49. A method according to any one of claims 40 to 48, wherein the subject is suffering from an inherited primary neutropenic disorder.
- 25 50. A method according to any one of claims 40 to 48, wherein the subject is suffering from an inherited primary or secondary defect of phagocytic cell function.
51. A method according to any one of claims 40 to 48, wherein the subject is suffering from an acquired defect of phagocytic cell function.
- 30 52. A method according to any one of claims 40 to 48, wherein the subject is at risk of or is suffering from nosocomial infection.
53. A method according to any one of claims 40 to 52, wherein the peptide is co-administered with interferon gamma.
- 35 54. A method of treating a subject in whom enhanced neutrophil and/or monocyte/macrophage function is desirable, the method comprising

administering to the subject a therapeutic amount of a peptide according to any one of claims 11 to 13.

55. A method according to claim 54, wherein the subject is suffering  
5 from a condition caused by an intracellular pathogen selected from the group consisting of: Mycobacteria, Chlamydia, Brucellae, Francisella, Pasteurellosis, Legionellosis, Histoplasmosis, Listeriosis, *Pneumocystis carinii* and *Trypanosoma cruzi*.

10 56. A method according to claim 55, wherein the subject is suffering from a condition caused by Mycobacteria.

57. A method according to claim 55 or 56, wherein the peptide is co-administered with an antibiotic agent.

15

58. A method according to claim 57, wherein the antibiotic agent is selected from the group consisting of: isoniazid, rifampin, pyrazinamide, ethambutol, rifabutin, azithromycin, clarithromycin, amikacin, cefoxitin and erythromycin.

20

59. A method according to claim 54, wherein the subject is suffering from cancer.

60. A method according to claim 59, wherein the peptide is co-administered with a cancer chemotherapy agent.

25

61. A method according to claim 60, wherein the cancer chemotherapy agent is selected from aminoglutethimide, amsacrine, azacitidine, busulfan; carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide,  
30 cytarabine HCl, dacarbazine, dactinomycin, deoxycoformycin, doxorubicin, etoposide, floxuride, fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, lomustine, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitoxantrone, plicamycin procarbazine HCl, fludarabine and 2-cyclodeoxyadenosine.

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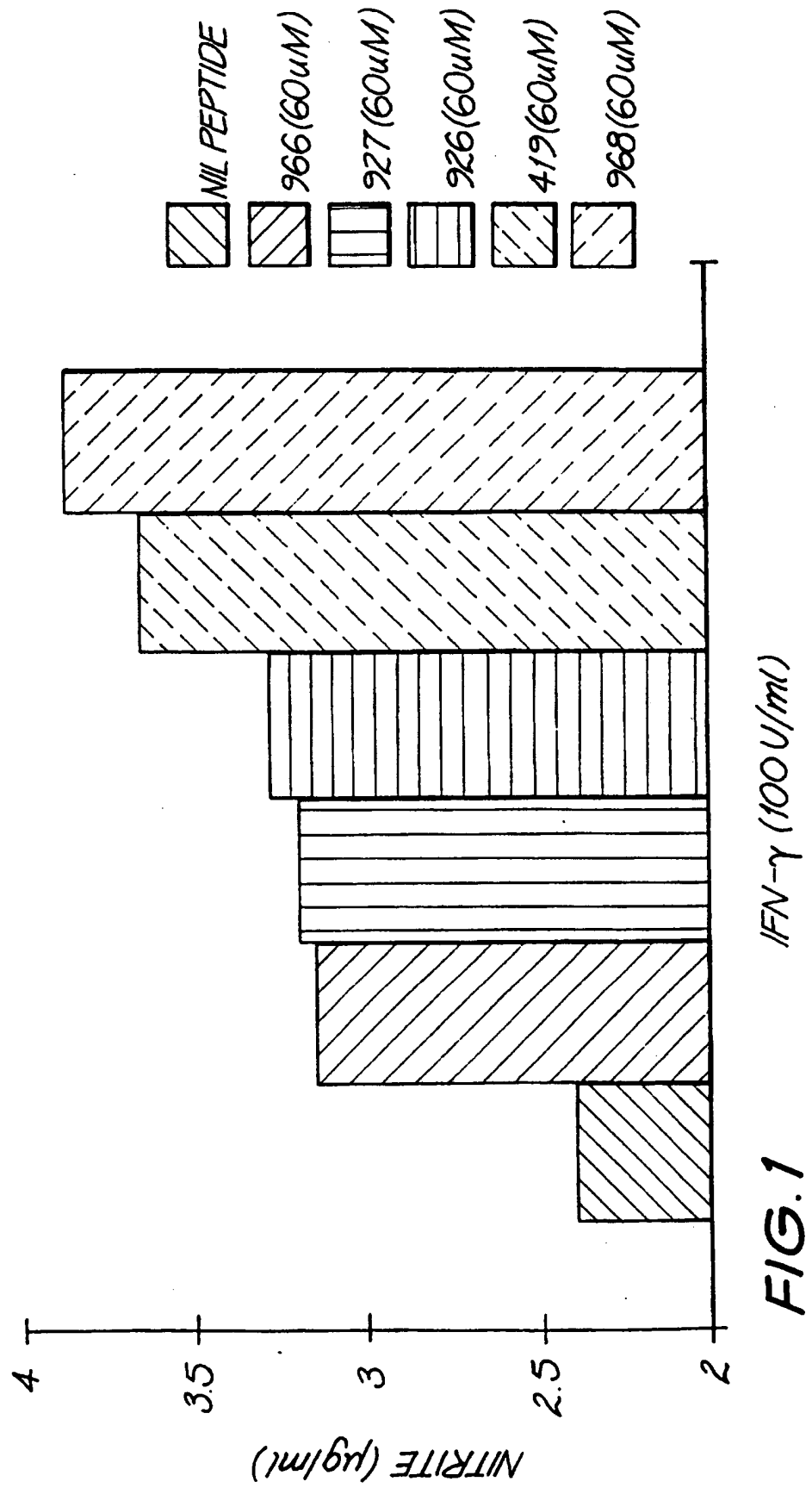
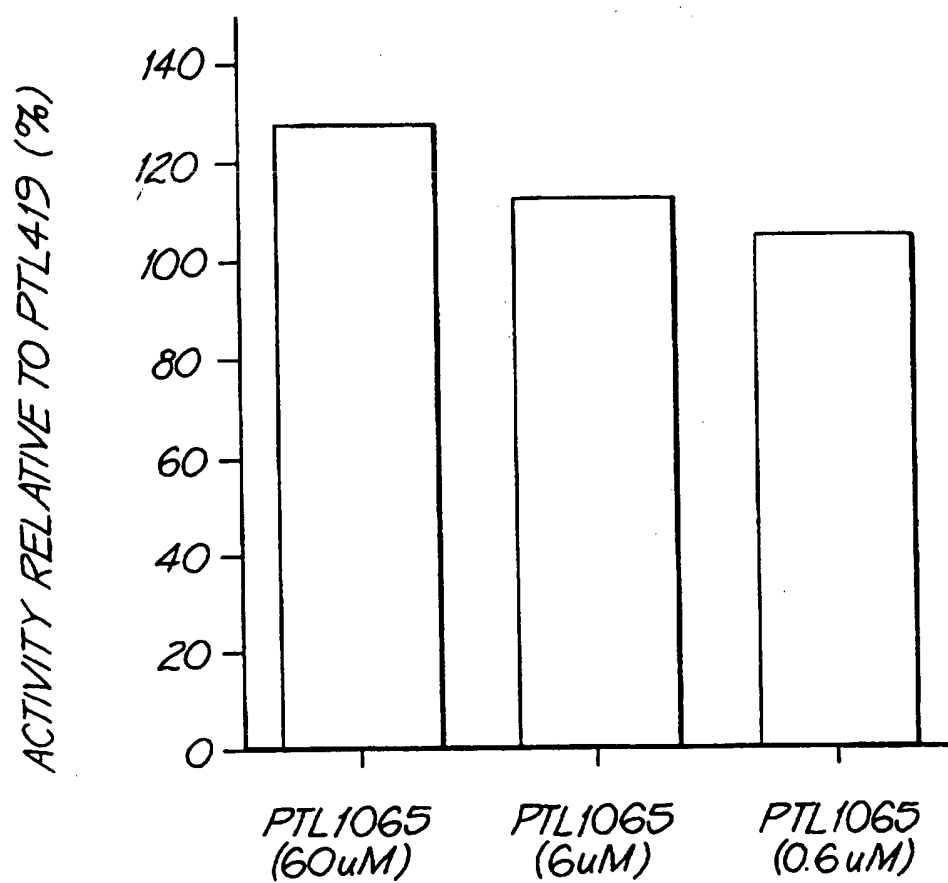


FIG. 1

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**FIG. 2**

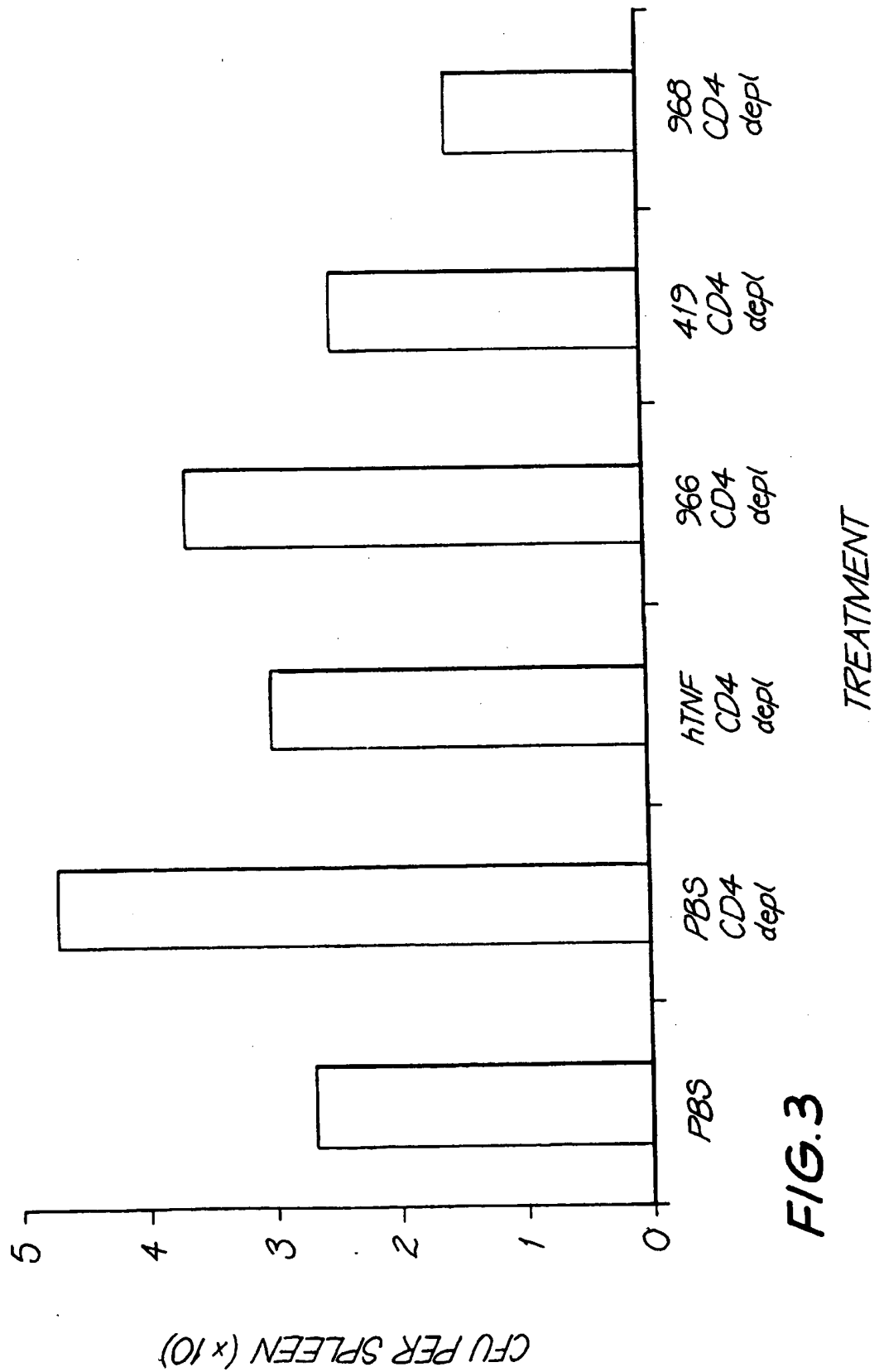
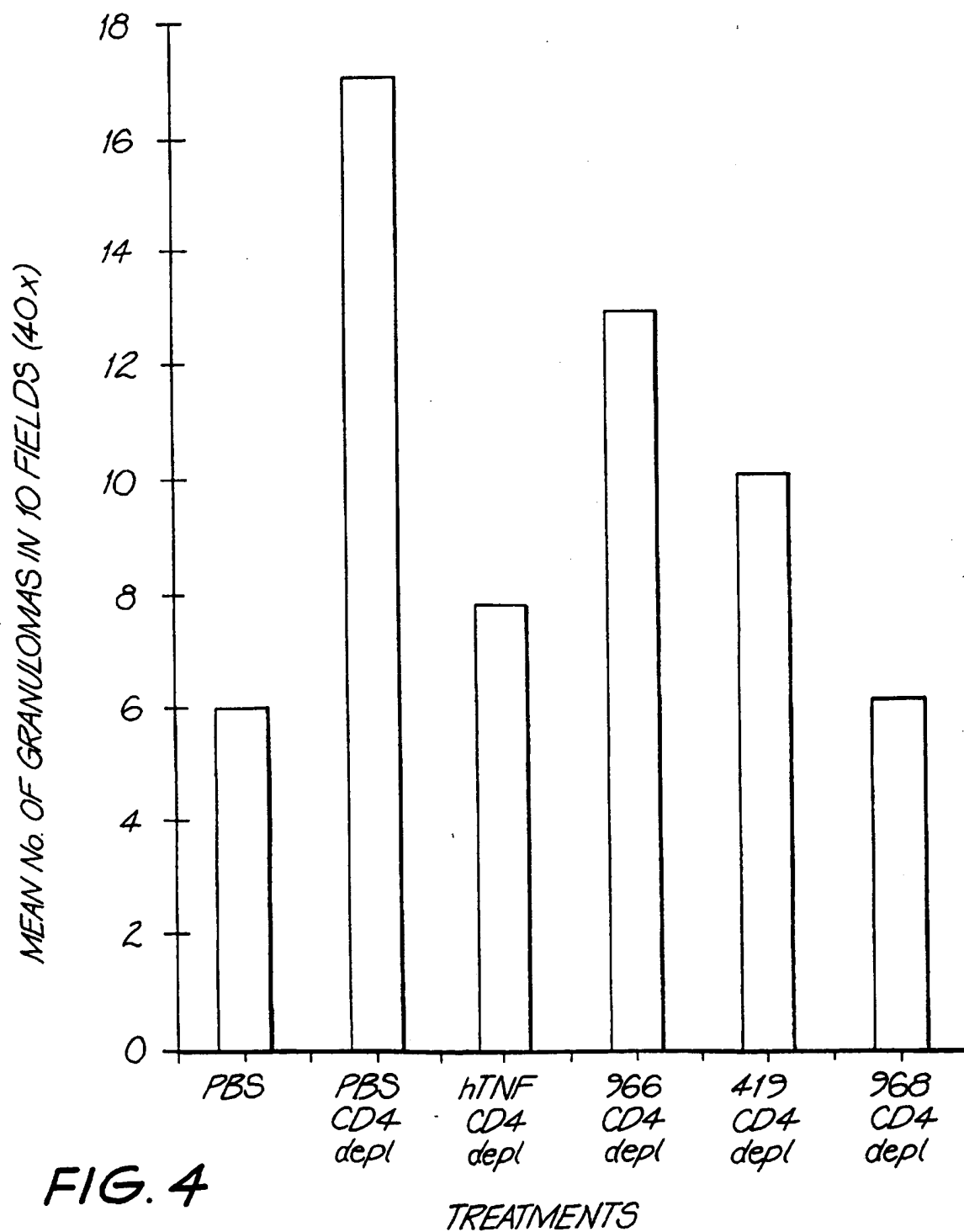


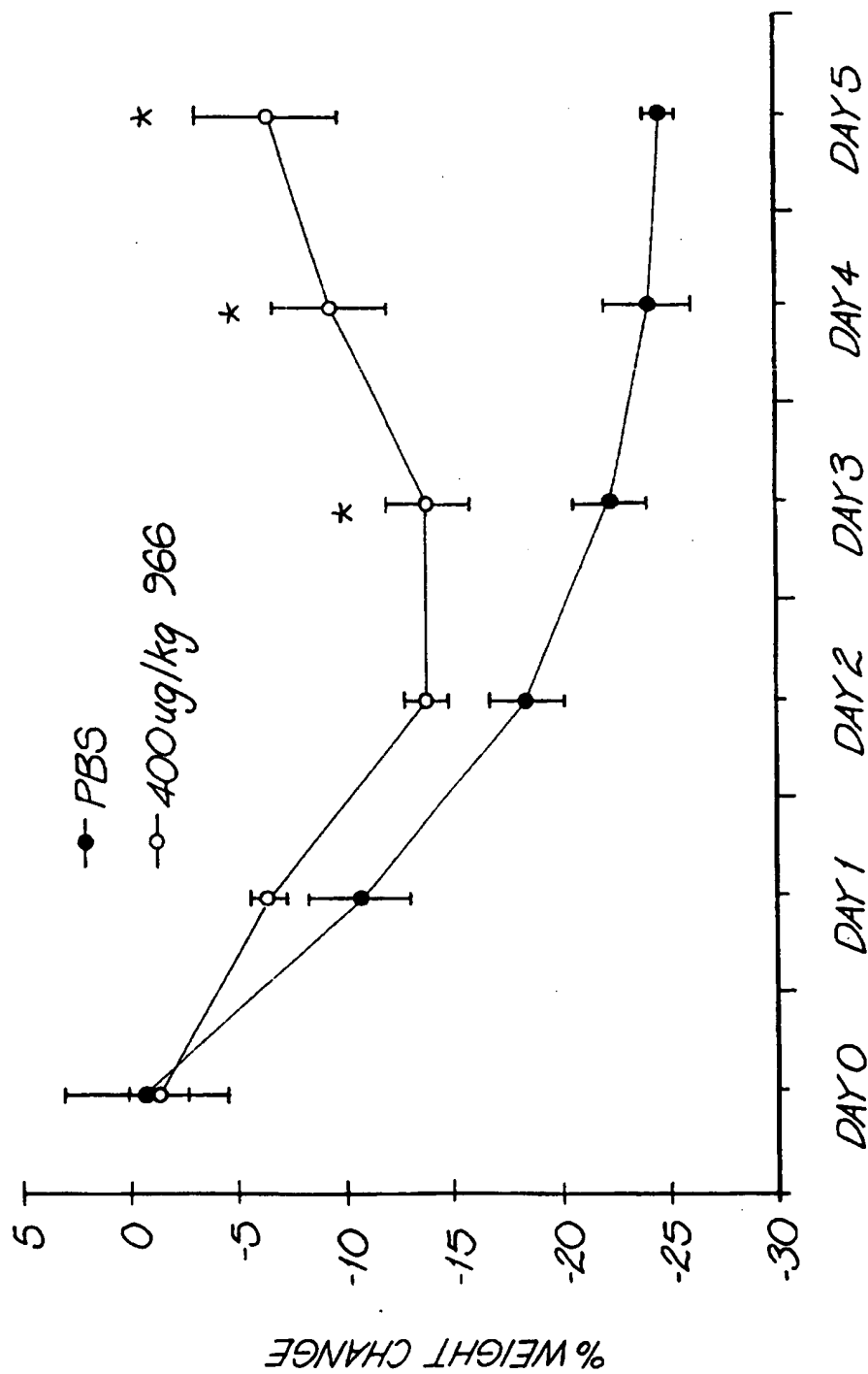
FIG. 3

62. A method according to claim 54 or 59 to 61, wherein the peptide is co-administered with G-CSF, GM-CSF, levamisole or other agents which stimulate increased white blood cell numbers.
- 5 63. A method according to any one of claims 49 to 56, wherein the peptide is co-administered with interferon gamma.

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DAYS POST INFECTION

FIG. 5

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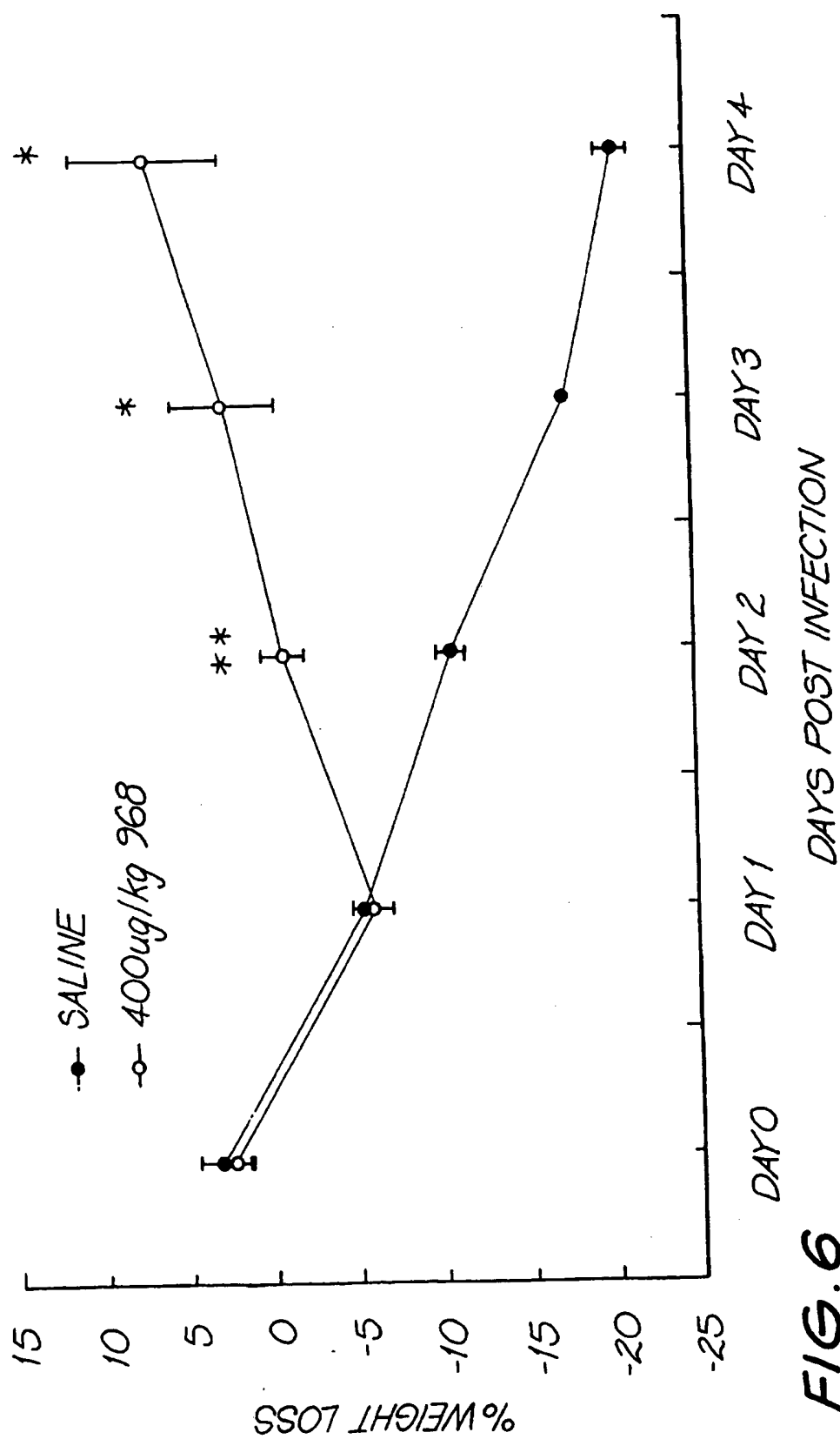


FIG. 6

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00395

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>																						
Int Cl <sup>B</sup> : C07K 7/06, 7/08, 7/10; A61K 37/02																						
According to International Patent Classification (IPC) or to both national classification and IPC																						
<b>B. FIELDS SEARCHED</b>																						
Minimum documentation searched (classification system followed by classification symbols)																						
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched																						
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN 'SER-THR-[ALAHIS]-VAL-[ALAGLYILELEUPHEPROSERTHRTRPVALBAL]-ILE-THR-[HISALA']																						
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																						
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																				
X	AU, B, 74762/91 (642487) (PEPTIDE TECHNOLOGY LTD) 19 September 1991 The whole document, see csg claim 1	1-63																				
X	AU, B, 44664/93 (676842) (PEPTIDE TECHNOLOGY LTD) 2 March 1995 The whole document	1-63																				
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex																						
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E"</td> <td>earlier document but published on or after the international filing date</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&amp;"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E"	earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family	"P"	document published prior to the international filing date but later than the priority date claimed		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																			
"E"	earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																			
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																			
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family																			
"P"	document published prior to the international filing date but later than the priority date claimed																					
Date of the actual completion of the international search 9 July 1997		Date of mailing of the international search report <b>21 JUL 1997</b>																				
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929		Authorized officer  <b>K.F. PECK</b> Telephone No.: (06) 283 2263																				



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00395

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Clinical Investigation Vol 95 No. 5 issued 1995 Kumaratilake et al "A Synthetic tumor necrosis factor-alpha. agonist peptide enhances human polymorphonuclear leukocyte mediated killing of Plasmodium falciparum in vitro and suppresses Plasmodium chabaudi infection in mice" p2315-2323, esp methods and figure 1	1-63

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00395

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	74762/91	CA	2078000	EP	519976	US	5587457
		WO	91/13908				
AU	44664/93	WO	96/18657	US	5527753		
							END OF ANNEX